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Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks?

Nigel E. Hussey, Sheldon F.J. Dudley, Ian D. McCarthy, Geremy Cliff, and Aaron T. Fisk

Abstract: Understanding the role of predators is challenging but critical for ecosystem management. For community dynamics, predator-specific size-based variation in diet, trophic position, and habitat use are rarely accounted for. Using two applied tools (stable isotopes and stomach content data), we examined inter- and intra-species ontogenetic variability in diet (stomach contents), trophic position (TP_{MA} for $\delta^{15}N$ and TP_{SCA} for stomach contents), and habitat use ($\delta^{13}C$) of two large sharks, the scalloped hammerhead (*Sphyrna lewini*) and the dusky (*Carcharhinus obscurus*). Stomach contents identified size-based and gender-specific shifts in diet indicating resource partitioning for and between species. Calculated TP for the two sharks varied by method, either TP_{MA} or TP_{SCA} and with species, size, and gender, but were complicated by differing baselines and broad functional prey groups, respectively. TP increased with size for *S. lewini*, but was low in large *C. obscurus* compared with small sharks. Size-based $\delta^{13}C$ profiles indicated habitat partitioning by sex in *S. lewini* and a movement to shelf edge foraging in large *C. obscurus*. These results demonstrate that predators exert proportional size-based effects on multiple components of the marine system that are further complicated by species- and gender-specific strategies.

Résumé : Comprendre le rôle des prédateurs représente un défi, mais c'est essentiel pour la gestion des écosystèmes. Dans les études de dynamique des communautés, on tient rarement compte des variations reliées à la taille et spécifiques aux prédateurs dans le régime alimentaire, la position trophique et l'utilisation de l'habitat. À l'aide de deux méthodes, les isotopes stables et l'analyse des contenus stomacaux, nous avons examiné la variabilité inter- et intraspécifique durant l'ontogenèse du régime alimentaire (contenus stomacaux), de la position trophique (TP_{MA} pour $\delta^{15}N$ et TP_{SCA} pour contenu stomacal) et de l'utilisation de l'habitat ($\delta^{13}C$) chez deux grands requins, le requin-marteau halicorne (*Sphyrna lewini*) et le requin obscur (*Carcharhinus obscurus*). Les contenus stomacaux permettent d'identifier des modifications dans le régime alimentaire reliées à la taille et spécifiques au sexe qui indiquent un partitionnement des ressources chez et entre les espèces. Les TP calculées pour les deux requins varient d'après la méthode utilisée, soit TP_{MA} ou TP_{SCA}, et aussi en fonction de l'espèce, de la taille et du sexe et elles sont compliquées respectivement par des lignes de base différentes et par des groupes fonctionnels de proies distincts. La TP augmente en fonction de la taille chez *S. lewini*, mais elle est basse chez les grands *C. obscurus* par comparaison aux petits requins. Les profils de $\delta^{13}C$ indiquent un partitionnement de l'habitat en fonction du sexe chez *S. lewini* et un déplacement vers le bord de la plate-forme chez les grands *C. obscurus*. Ces résultats démontrent que les prédateurs exercent des effets proportionnels à leur taille sur plusieurs compartiments du système marin, qui sont de plus compliqués par des stratégies spécifiques à l'espèce et au sexe.

[Traduit par la Rédaction]

Introduction

Body size has long been recognized to have an important influence on the structural and functional complexity of aquatic food webs (Elton 1927). As predators are typically larger than their prey, trophic position (TP) is predicted to increase with body size (Cohen et al. 1993). Size as a surrogate measure of TP has therefore been used as a tool to examine human-induced impacts on community structure (Pinnegar et al. 2002; Dulvy et al. 2004), alterations in trophic linkages (Pauly et al. 1998), and (or) cascading effects of predator removal (Myers et al. 2007).

Large marine predators are typically mobile, undertaking large-scale movements and rapidly expanding home range with size (Bonfil et al. 2005). As a consequence, size-based variation in habitat use and relative TP of these predators

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can modify inferences on food web structure and assessments of community structure over time (Layman et al. 2005). Specifically, for analysis of community trophic structure (Jennings et al. 2001; Layman et al. 2005), (*i*) within-species size-based variation and (*ii*) associated size-based habitat shifts are not clearly defined and (or) generally accounted for.

Diet and trophic ecology of fish have been traditionally studied using stomach content analysis (Hyslop 1980; Cortés 1999). However, for sharks, as with many large, highly mobile predatory fish, stomach content analysis is hindered by insufficient sampling of all size classes and incomplete sampling across the geographic range of the animal both in temporal and spatial domains. More recently, the stable isotopes of nitrogen ($^{15}N/^{14}N$ expressed relative to a standard as $\delta^{15}N$) have been proven as a valuable tool in elucidating trophic relations in aquatic food webs (Vander Zanden et al. 1997; Post 2002) and have recently been applied in the study of sharks (Fisk et al. 2002; Estrada et al. 2006; Hussey et al. 2011).

The conservative fractionation of carbon ($^{13}C/^{12}C$ expressed relative to a standard as $\delta^{13}C$) between primary producers and top-level predators can also provide a tool to track animal movements between regions and to define habitat use (Hobson 1999; Rubenstein and Hobson 2004). When considering the inherent difficulties and expense involved in the examination of fine-scale movement patterns of predators through archival or satellite tracking telemetry (Block et al. 2001), there is a requirement to target specific life stages and sexes to provide the required data for effective management of exploited–threatened species. Size-based $\delta^{13}C$ data may provide the necessary coarse resolution movement profiles of little known species to guide the size-based application of telemetry methods, consequently improving the efficiency of yielded results.

The aim of the current study was threefold: (*i*) to examine size-based and gender-specific variations in diet, TP, and habitat use of two large marine predators, the scalloped hammerhead (*Sphyrna lewini*) and dusky shark (*Carcharhinus obscurus*), by combining stomach content analysis (from a long-term archived database) and stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) of muscle tissue; (*ii*) to quantitatively compare and contrast TP calculated using these two commonly applied TP techniques; and (*iii*) to assess the utility of $\delta^{13}C$ as an indicator of coarse resolution ontogenetic movement profiles in these sharks, because $\delta^{13}C$ values have been shown to vary systematically across the geographic range of these species in South Africa (Hill et al. 2006; Hill and McQuaid 2008). With documented worldwide population declines of large sharks (Myers and Worm 2003) and gender and (or) size classes targeted by area-specific fishing practices (Mucientes et al. 2009), a more thorough understanding of species-specific trophic dynamics and movement patterns of large predators with relation to changes in body size is required (Layman et al. 2005). Concurrently, the increase in studies utilizing stable isotopes in sharks, coupled with current literature and archival stomach content data sets, requires an understanding of the issues associated with calculating TP and how the TP results vary by method.

Materials and methods

Location and sampling

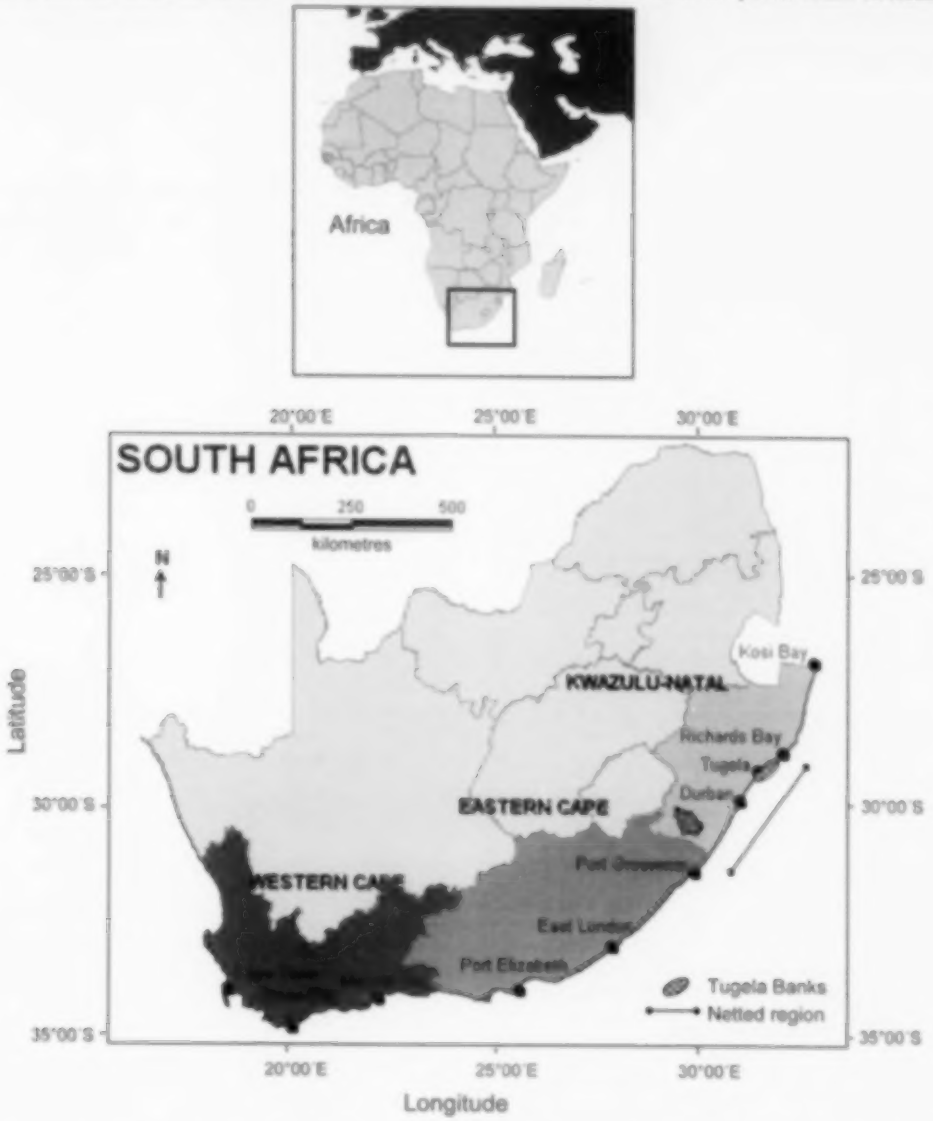
All scalloped hammerhead and dusky sharks were sampled from animals incidentally caught in beach protection nets in KwaZulu-Natal (KZN), South Africa, with the exception of five juvenile scalloped hammerhead sharks sampled from commercial trawl fisheries on the Tugela Banks (Fig. 1). Beach net installations were set parallel to the shoreline, approximately 300–500 m from the beach in 10–14 m (depth) of water. All shark mortalities that appeared to be recent mortalities and in good condition were retrieved, transported to the KwaZulu-Natal Sharks Board (KZNSB) laboratory, and stored frozen (–20 °C) until dissection. On arrival at the laboratory, basic data on species, sex, and morphological measurements, including precaudal length (PCL, cm), were recorded. For establishing the baseline inshore (coastal) and offshore (pelagic) stable isotope values of the KZN marine system, the grooved mullet (*Liza dumerili*) was sampled in shallow coastal waters off Durban, and whale sharks (*Rhincodon typus*) and devil rays (*Mobula* sp.) were sampled from beach strandings and KZNSB net catches, respectively.

Stomach content analysis

The diet of the scalloped hammerhead and dusky shark were quantified using stomach content data accessed from the KZNSB archived database (1983–2006). A total of 1018 scalloped hammerhead and 900 dusky sharks contained stomach contents and were included in the analysis. For specific details on species catch rates and seasonality of capture, refer to Dudley et al. (2005) and de Bruyn et al. (2005). For each shark, the complete stomach was removed, prey items identified to the lowest possible taxon, and the number and mass (to 0.1 g) of each prey item recorded. To summarize the stomach content data, all prey items were grouped to family level and then further grouped into eight functional prey groups, defined as elasmobranch, teleost, cephalopod, crustacean, mollusc, mammal, bird, and miscellaneous items (Cortés 1999). The percent mass contribution (% mass) of each functional prey group to the diet of the two shark species was then calculated. To examine size-based and gender shifts in diet for the two species, the data were divided by sex and into three size classes for scalloped hammerhead sharks (small, <110 cm; medium, 110–140 cm; and large, >140 cm) and for dusky sharks (small, <100 cm; medium, 100–209 cm, and large, ≥210 cm). Large female dusky sharks were further divided into reproductively inactive (Linact), pregnant (Lpreg), and postpartum (Lpost) sharks. The size classes selected approximate to maturity stage and relate to species-specific life strategies (Compagno 1984).

The % mass contribution of the functional prey groups for the two species of sharks divided by sex and the predefined size classes were (*i*) plotted in histogram form and (*ii*) subjected to nonmetric multidimensional scaling (MDS) ordination to infer the overall influence of species, sex, and body size on diet composition. Accepting the limited number of prey items–types per individual shark stomach, dietary data for groups of individual animals (approximately 10 per group) were randomly pooled within each size class, herein referred to as dietary samples (White et al. 2004). Prior to MDS ordination, the dietary samples were ARCSIN-transformed, and a similarity matrix was constructed using the Bray–Curtis similarity coefficient in PRIMER (PRIMER-E Ltd., Ivybridge, UK). A one-way analysis of similarities (ANOSIM) test was then employed to determine if statistical differences in diet

Fig. 1. The study region encompassing the area protected by beach protection nets and the Tugela Banks in KwaZulu-Natal, South Africa. The netted region is currently composed of 38 individual net installations (see Dudley et al. 2005 for specific details on locations).



composition occurred between the two species. Individual ANOSIM tests were then undertaken to examine the diet of each species (male and female separately) by size. The ANOSIM test generates a global R statistic that provides a measure of the magnitude of difference among groups. The global R statistic ranges from –1 to +1, with 0 indicating no difference in groups, while +1 is complete separation.

Stable isotope analysis

All net-caught scalloped hammerhead (n = 38: size range: 52–223.4 cm PCL) and dusky (n = 63; size range: 70–280 cm PCL) sharks were sampled between 2005 and 2008. Trawl fishery-derived scalloped hammerhead (n = 5; size range: 38–58 cm PCL) were sampled in 2006. Baseline species, grooved mullet and devil ray – whale sharks were sampled between 2007 and 2009. White muscle tissue was excised from the muscle block anterior to the first dorsal fin adjacent to the vertebral column in sharks, from the wing-body cavity margin in rays, and from the anterior dorsal section in teleost fish. All samples were stored frozen (–20 °C). The muscle tissue was then freeze-dried and homogenized. Lipid extraction of all samples was undertaken by agitating the dried powdered muscle tissue in a 2:1 chloroform–methanol solution for 24 h. The tissue and solvent were then filtered and the resulting residue–filter paper dried at 60 °C for 48 h to evaporate the remaining solvent. Lipid extraction was undertaken because of the bias of soluble urea in shark muscle tissue (Hussey et al. 2010*a*; N.E. Hussey, unpublished

data). Between 400 and 600 μg of lipid extraction tissue was weighed into tin capsules, and stable carbon and nitrogen isotope ratios were provided from a continuous flow isotope ratio mass spectrometer (Finnigan MAT Delta^{plus}, Thermo Finnigan, San Jose, California, USA).

Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

$$(1) \qquad \delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{15}N or ^{13}C and R is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The standard reference material was Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen for N_2. The analytical precision for $\delta^{15}N$ was <0.22‰ and for $\delta^{13}C$ was <0.24‰ based on more than 100 analyses of a NIST standard 8414 (bovine muscle) across multiple runs and was 0.14‰ for $\delta^{15}N$ and 0.05‰ for $\delta^{13}C$ based on a single run of NIST standard sucrose (n = 13) and ammonium sulphate (n = 13).

Small scalloped hammerhead and dusky sharks with umbilical scar data were excluded from the following analyses because of maternal influence on stable isotope signatures (Olin et al. 2011). Size-based $\delta^{15}N$ profiles for each species were plotted and regression models fitted to the data by species and sex.

TP estimation from stomach contents and $\delta^{15}N$

To facilitate a comparison between methods of calculating TP, calculations were based on stomach contents (TP_{SCA}) and $\delta^{15}N$ (TP_{SIA}). Dietary samples for each shark species in conjunction with the estimated TP of functional prey groups were used to calculate TP_{SCA} using the following equation (Cortés 1999):

$$(2) \qquad TP_{SCA} = 1 + \left(\sum_{i=1}^{n} p_i \times TP_i\right)$$

where TP_{SCA} is the trophic position of the species in question, p_i is the proportion of each functional prey group i in the total diet (expressed as % mass), and TP_i is the trophic position for each functional prey category. TP of functional prey groups were taken directly from Cortés (1999), who assessed the TP of a wide range of shark species using stomach contents, and were defined as follows: elasmobranch (3.65), teleost (3.24), cephalopod (3.2), crustacean (2.52), mollusc (2.1), bird (3.87), and mammal (4.02). The miscellaneous functional prey group (Appendix A) was excluded from all trophic-level calculations.

To determine a measure of shark TP using $\delta^{15}N$ (TP_{SIA}) and accepting that large sharks are highly mobile and move between both coastal and offshore environments, we used a two-source food web model according to Post (2002):

$$(3) \qquad TP_{SIA} = \lambda + \{\delta^{15}N_{shark} - [\delta^{15}N_{base1} \times \alpha + \delta^{15}N_{base2} \times (1 - \alpha)]\}/\Delta^{15}N$$

where λ is the TP of the selected base consumers (TP = 3, zooplanktivorous fish), $\delta^{15}N_{base1}$ is the value of the coastal base consumer (the grooved mullet (mean ± standard deviation, SD): 12.63‰ ± 1.47‰; n = 5), $\delta^{15}N_{base2}$ is the value of the pelagic base consumer (the whale shark – devil ray (mean ± SD): 9.86‰ ± 0.45‰; n = 10), and α is the calculated proportion of the nitrogen in the consumer derived from the base of the coastal food web ($\delta^{15}N_{base1}$):

$$(4) \qquad \alpha = (\delta^{13}C_{shark} - \delta^{13}C_{base2})/(\delta^{13}C_{base1} - \delta^{13}C_{base2})$$

The $\delta^{13}C_{base1}$ of the grooved mullet was –13.23‰ ± 1.02‰ and $\delta^{13}C_{base2}$ of the whale shark – devil rays was –17.14‰ ± 0.3‰, which provide the expected negative $\delta^{13}C$ gradient between coastal and pelagic food webs (Post 2002; Hill et al. 2006). The grooved mullet inhabits shallow coastal areas, while devil rays and whale sharks are predominantly pelagic. To enable accurate inferences on TP within food webs requires knowledge of species-specific diet tissue discrimination factors ($\Delta^{15}N = \delta^{15}N_{shark} - \delta^{15}N_{food}$). A $\Delta^{15}N$ value of 2.3‰ for large sharks was used according to Hussey et al. (2010*a*, 2010*b*). A general linear model (GLM) with adjusted Bonferroni pairwise comparisons was used to examine the effects and interactions of sex, size, and method of calculating TP (TP_{SCA} and TP_{SIA}) on derived TP values for both shark species.

Habitat: size-based $\delta^{13}C$ profiles

To interpret regional $\delta^{13}C$ movement or residency patterns of the two shark species over ontogeny, the following assumptions were made: (*i*) The range of expected $\delta^{13}C_{shark}$ values in coastal waters were predicted using the mean (±1 SD) $\delta^{13}C$ values for small scalloped hammerhead (~60–100 cm PCL) and dusky (~100–140 cm PCL) sharks that have known nursery grounds on the continental shelf in KZN (Fennessy 1994; Hussey et al. 2009) and are regularly caught in beach protection nets (Dudley et al. 2005; de Bruyn et al. 2005). These animals therefore spend the majority of this life stage, over the tissue integration period, on the continental shelf. (*ii*) Distinct systematic latitudinal and inshore–offshore gradients in $\delta^{13}C$ occur off South Africa. With increasing distance from KZN to the Western Cape, there is a marked increase in $\delta^{13}C$ in coastal waters (Fig. 1; Hill et al. 2006; Hill and McQuaid 2008). Measurements of suspended particulate matter in offshore waters were substantially depleted in $\delta^{13}C$ compared with coastal waters (Hill et al. 2006).

Size-based $\delta^{13}C$ profiles were plotted for each species. Large sharks may be highly mobile, undertaking large unidirectional migrations but also rapid return migrations (Bonfil et al. 2005). Considering estimated turnover on carbon isotope ratios in muscle tissue (390–540 days; MacNeil et al. 2006), the identification of distinct size-based $\delta^{13}C$ profiles would only be expected if sharks remained resident in core isotopically distinct environments for extended periods of time.

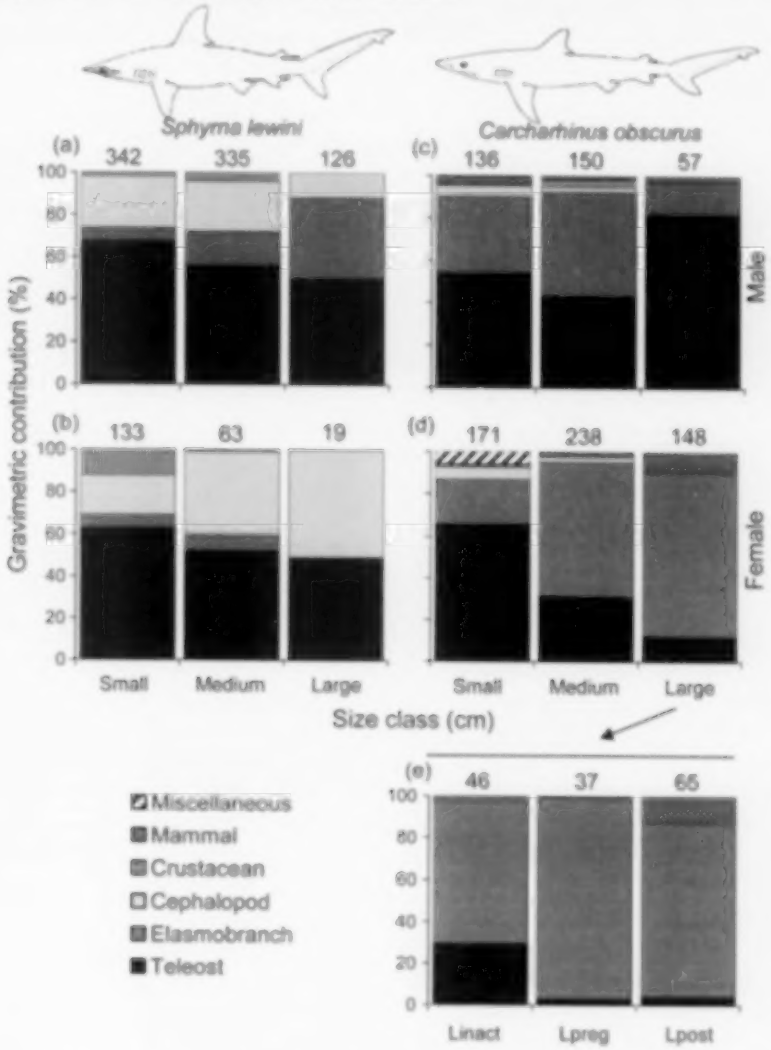
Results

Multivariate analysis of stomach content data

The overall % mass diet composition of the scalloped hammerhead shark was significantly different from that of the dusky shark (R statistic = 0.611; p = 0.001). Additionally, size-based shifts in diet were observed for male and female sharks of both species (Figs. 2 and 3; see Appendix A for detail of stomach contents).

For scalloped hammerhead sharks, small sharks of both sexes fed predominantly on teleosts. With increasing size, males included more elasmobranchs in the diet, while cepha-

Fig. 2. Percent mass (% mass) contribution of functional prey categories defined by Cortés (1999) to the diet of scalloped hammerhead (*a*, *b*) and dusky (*c*, *d*) sharks by size class and sex. (*e*) Large female dusky sharks are further subdivided into inactive (Linact), pregnant (Lpreg), and postpartum animals (Lpost). Numbers indicate sample sizes per size class.



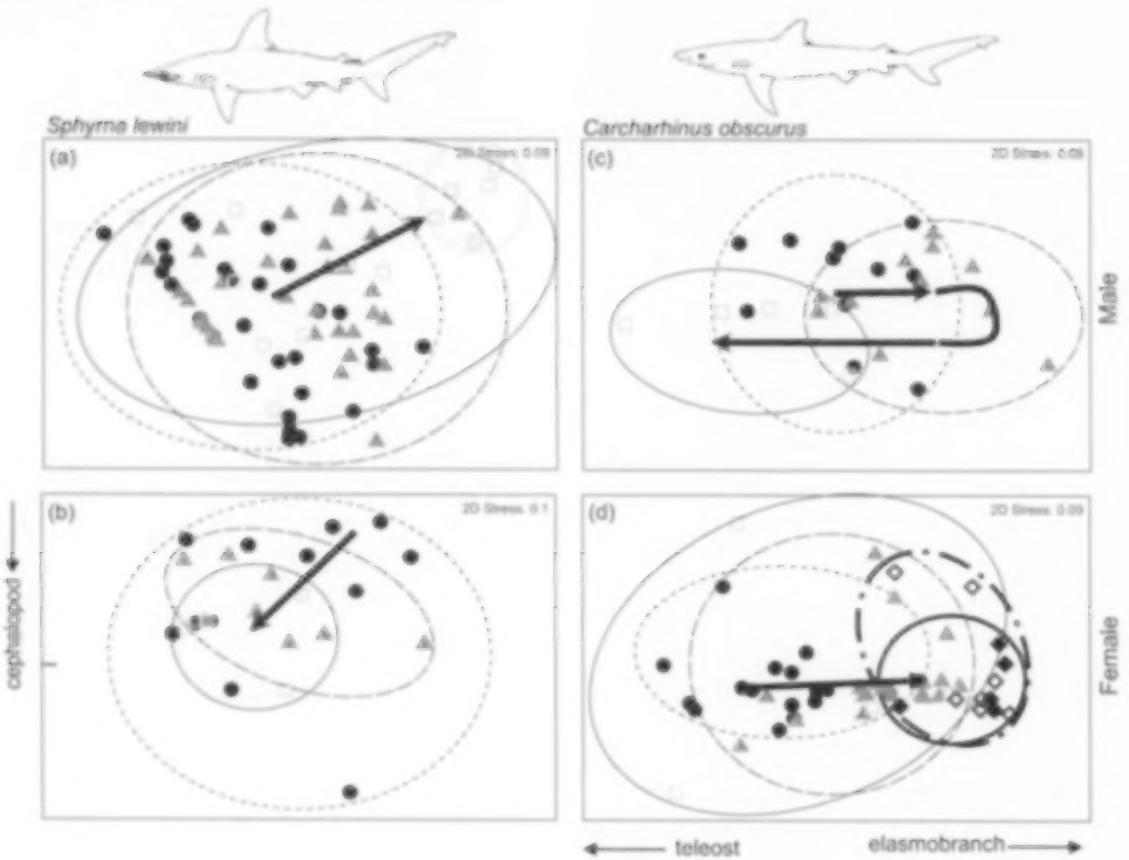
lopods dominated the diet of large females (Fig. 2; Appendix A, Table A1). MDS ordination of the male diet data found that several dietary samples of large animals were located to the right of the plot frame, identifying the importance of elasmobranchs (Fig. 3; Appendix A, Table A1). For females, medium and large sharks were more highly clustered within the MDS plot, indicating a transition to a cephalopod- and teleost-dominated diet (Fig. 3; Appendix A, Table A1).

The diet of small male and female dusky sharks was also dominated by teleosts but included an elasmobranch component (Fig. 2; Appendix A, Table A2). In contrast with scalloped hammerhead sharks, large male dusky sharks fed on teleosts, while the diet of large female sharks, particularly pregnant and postpartum animals, consisted of elasmobranchs (Fig. 2; Appendix A, Table A2). MDS ordination of the male dusky shark dietary samples found a clear transition in diet between the three size classes (Fig. 3). For female dusky sharks, MDS ordination found that data points were located further to the right of the plot frame with increasing size, highlighting the importance of elasmobranchs in the diet (Fig. 3). Pregnant–postpartum female data were highly clustered to the right, while nonactive–immature females exhibited a high degree of overlap with all size classes (Fig. 3). ANOSIM pairwise comparisons for each sex found that the diet of different size class male and female dusky sharks were significantly different (Fig. 3).

Size-based $\delta^{15}N$ profiles

For scalloped hammerhead sharks, males showed a significant increase in $\delta^{15}N$ with increasing PCL; accepting the small sample, the female increase was not significant (Fig. 4; $F_{[1,23]} = 56.84$, $p < 0.0001$ and $F_{[1,6]} = 4.71$, $p = 0.073$;

Fig. 3. Nonmetric multidimensional scaling (MDS) ordination of the percent mass (% mass) dietary samples of scalloped hammerhead (*a*, *b*) and dusky (*c*, *d*) sharks for each sex, by size class. For scalloped hammerhead and dusky sharks, black circles indicate small, grey triangles are medium, and open squares are large reproductively inactive sharks. For female dusky sharks, black diamonds indicate pregnant and open diamonds show postpartum sharks. Ellipses mark the outer bounds of dietary samples for small (thin short dashed lines), medium (thin long dashed lines), large–inactive (thin solid lines), pregnant (thick solid lines), and postpartum (thick dot-dashed lines) size classes. Arrows within the MDS indicate the size-based trends in diet. The arrows associated with functional prey groups (*x* and *y* axes) identify major components in the diet. For male scalloped hammerhead sharks, the shaded grey circle highlights large sharks focused on an elasmobranch diet. Significant ANOSIM pairwise comparisons for each species and sex by size are shown with the global *R* value and level of significance (***, $p < 0.001$; **, $p < 0.01$; and *, $p < 0.05$).



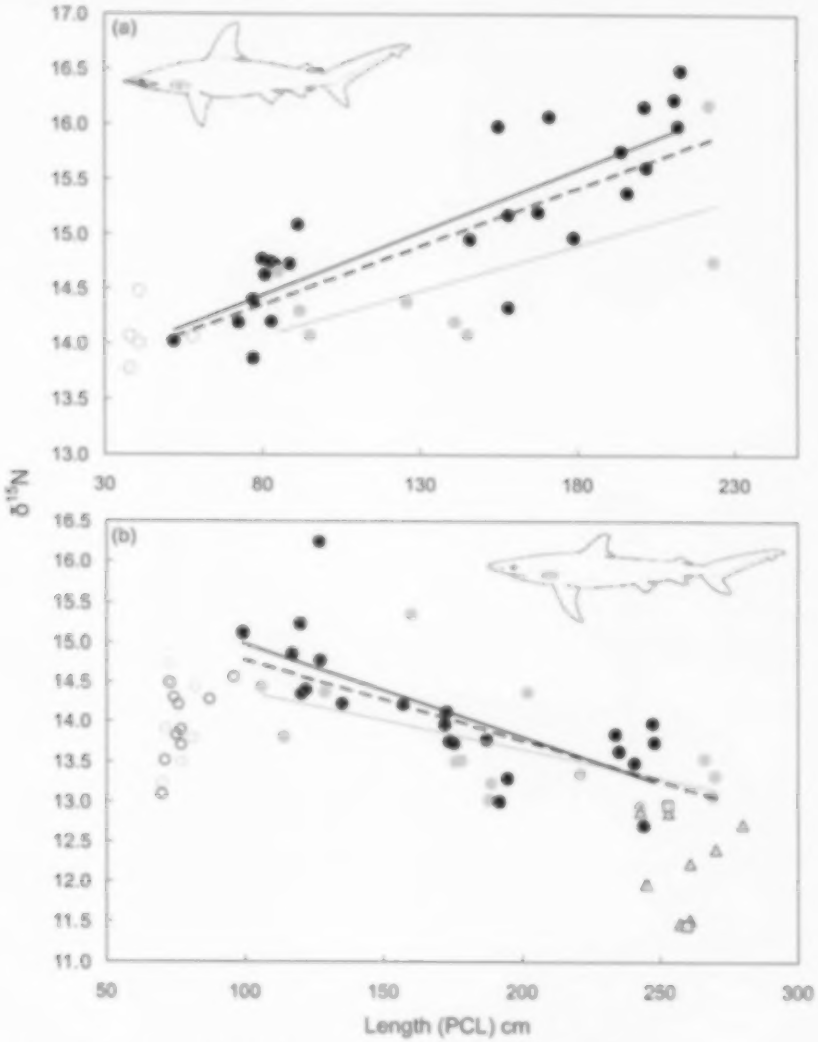
male and female, respectively). Excluding pregnant and postpartum animals from the analysis, a significant negative linear relationship between $\delta^{15}N$ and PCL was observed for both sexes of dusky shark ($F_{[1,20]} = 23.90$, $p < 0.0001$ and $F_{[1,12]} = 6.16$, $p = 0.029$; male and female, respectively). Pregnant and postpartum dusky sharks had lower $\delta^{15}N$ values than inactive large sharks ($t_{[11]} = -4.11$, $p = 0.002$; Fig. 4), but exhibited a degree of variability, with $\delta^{15}N$ values ranging from 11.4‰ to 13.0‰.

Trophic-level estimation: stomach contents and $\delta^{15}N$

Mean TP_{SCA} (±1 standard error, SE) of scalloped hammerhead and dusky sharks for both sexes and all size class combined were 4.3 ± 0.01 and 4.5 ± 0.02, respectively. Mean TP_{SIA} of all scalloped hammerhead and dusky sharks were 4.7 ± 0.05 and 4.3 ± 0.04, respectively.

For male scalloped hammerhead sharks, TP_{SCA} and TP_{SIA} increased with increasing animal size, whereas the TP_{SCA} value for female sharks was similar across size classes, but

Fig. 4. $\delta^{15}N$ ontogenetic profiles of (*a*) scalloped hammerhead and (*b*) dusky sharks by sex (black circles represent males, grey circles represent females); for dusky sharks, triangles represent pregnant sharks, squares represent postpartum sharks, and open circles represent neonatal animals with umbilical scar. Linear regression models were fitted to both scalloped hammerhead and dusky sharks by sex (solid black line: male; solid grey line: female) and for sex data combined (broken line).



TP_{SIA} increased with size (Fig. 5). Intrasize class variability between calculated TP_{SCA} and TP_{SIA} of both sexes of scalloped hammerhead sharks was evident (Fig. 5). A GLM on the scalloped hammerhead TP data (size class 1 and 3 animals) found that size and TP method had a significant effect (variance explained: 71.2%; Table 1; Fig. 5). The trend of calculated TP_{SCA} for male dusky sharks was similar across size classes, while TP_{SIA} showed a minor decline with increasing size class (Table 1; Fig. 5). For female dusky sharks, clear differences in estimated TP by TP_{SCA} and TP_{SIA} were observed among the Linact, Lpreg, and Lpost sharks (Fig. 5). A GLM testing only medium and Linact sharks found there was no significant effect of sex, size, or TP method on calculated TP (variance explained: 10.3%; Table 1).

Size-based $\delta^{13}C$ profiles

The $\delta^{13}C$ values of small scalloped hammerhead sharks between 70 and 100 cm, which were primarily males, were highly clustered with minimal variability (range: −15.69‰ to −15.21‰; mean ± SE: −15.41‰ ± 0.16‰; Fig. 6). Accepting the small sample size, the $\delta^{13}C$ values of male and female scalloped hammerhead sharks between 120 and 160 cm PCL indicated sexual segregation with a difference of ~2.5‰ (Fig. 6). The $\delta^{13}C$ signatures of male scalloped hammerheads >160 cm PCL then declined to a point where the signatures of both sexes >200 cm were similar (Fig. 6). For dusky sharks <160 cm PCL, $\delta^{13}C$ values were similar to those of small scalloped hammerhead sharks but were more variable. Most dusky sharks >200 cm PCL of both sexes were depleted in ^{13}C relative to predicted KZN continental shelf signatures (Fig. 6). Preg-

Fig. 5. Mean calculated trophic position (±1 standard error, SE) by diet (TP_{SCA}, striped bars) and $\delta^{15}N$ (TP_{SIA}, solid bars) for each sex and *size* class of the scalloped hammerhead shark (black bars; *a*, *b*) and dusky shark (grey bars; *c*, *d*).

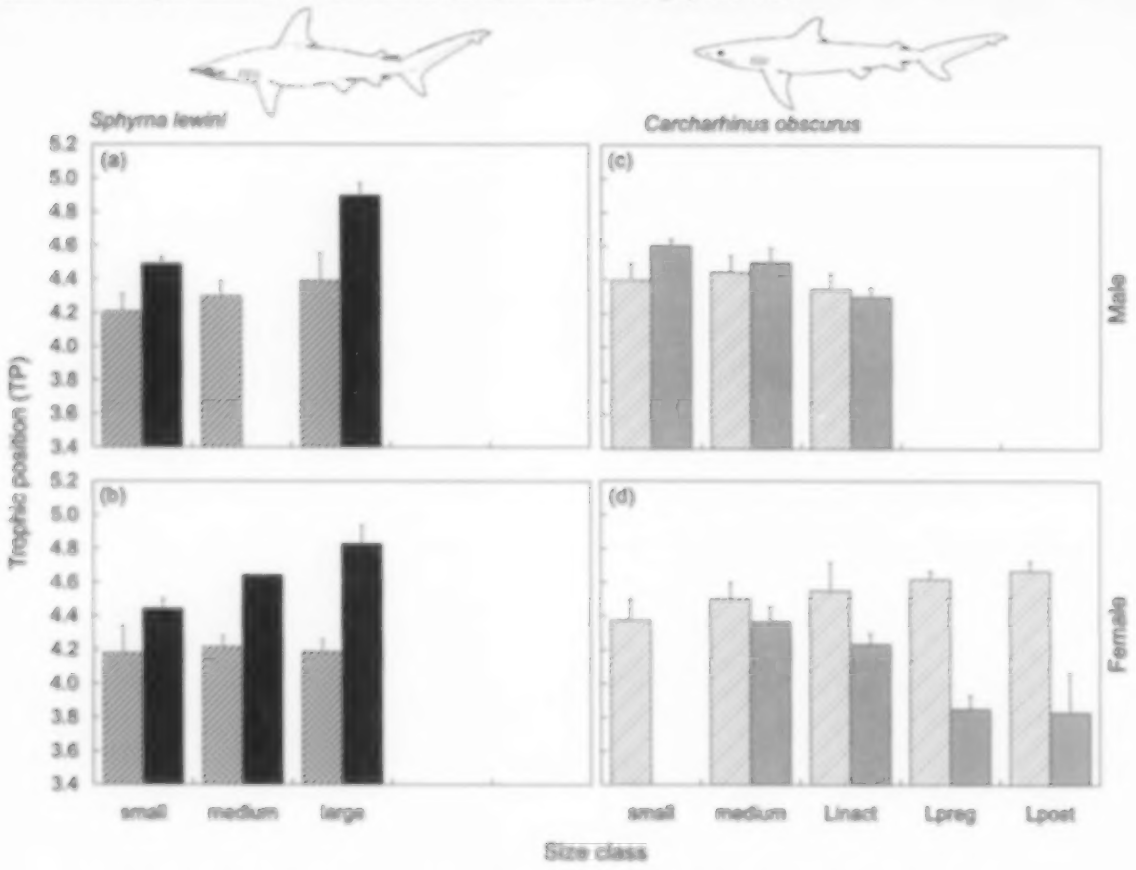


Table 1. General linear models (GLMs) testing the effects of size, sex, method of calculating trophic position (TP_{SCA} and TP_{SIA}), and associated interactions on estimated TP for the scalloped hammerhead and dusky shark.

	Scalloped hammerhead				Dusky			
Source	df	MS	*F*	*p*	df	MS	*F*	*p*
Size	1	0.865	33.83	**<0.0001**	1	0.140	3.45	0.067
Sex	1	0.116	4.52	0.036	1	0.003	0.08	0.782
Method	1	2.586	101.09	**<0.0001**	1	0.183	4.51	0.037
Size × sex	1	0.032	1.23	0.270	1	0.050	1.23	0.271
Size × method	1	0.335	13.10	**<0.0001**	1	0.078	1.93	0.169
Sex × method	1	0.013	0.49	0.484	1	0.210	5.18	0.026
Size × sex × method	1	0.019	0.75	0.388	1	0.006	0.15	0.702
Residual	86	0.026			69	0.041		

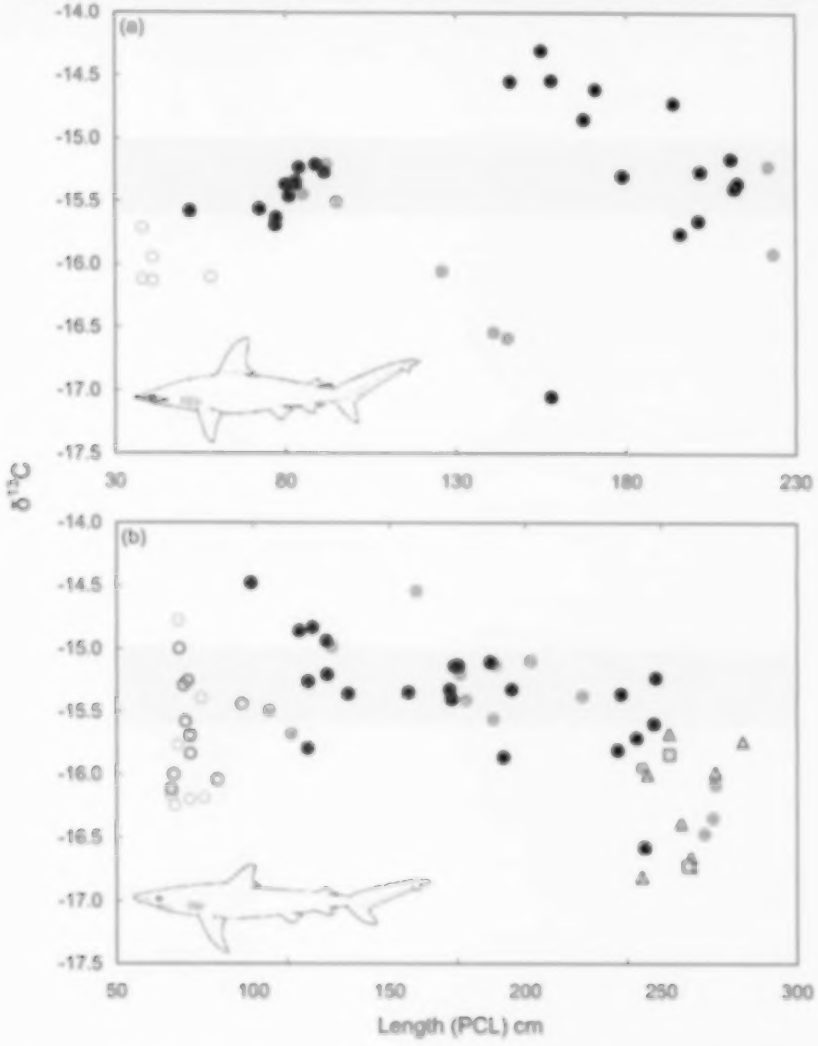
Note: A criterion for significance of $p < 0.01$ was set for all tests; bold font indicates significant factors and interactions.

nant and postpartum *C. obscurus* $\delta^{13}C$ values were more depleted than large inactive male and female sharks (min.: −16.8‰ and max.: −15.7‰).

Discussion

Knowledge of size-based effects of large marine predators is vital to our understanding of ecosystem structure and function and to predict the effects of species removal. To date, our principal understanding of the TP of sharks has stemmed from stomach content analysis as a stand alone proxy (Cortés 1999) and initial work investigating the application of stable isotopes (Fisk et al. 2002; Estrada et al. 2003, 2006). These data provide a comparative analysis of stomach content analysis and $\delta^{15}N$ and $\delta^{13}C$ data for two large sharks and provide empirical evidence for size-based and gender driven variations in diet, TP, and habitat use. Accepting the complexities of calculating TP using both TP_{SCA} and TP_{SIA}, a combined

Fig. 6. $\delta^{13}C$ ontogenetic profiles of (*a*) scalloped hammerhead and (*b*) dusky sharks by sex (black circles represent males, grey circles are females, and open circles are neonatal animals with umbilical scar); for dusky shark, triangles are pregnant sharks, and squares are postpartum sharks. Grey bar depicts the predicted $\delta^{13}C$ range of the KwaZulu-Natal (KZN) coastal habitat of scalloped hammerhead and dusky sharks.



method approach was required to better understand these size-based interactions.

Size-based trophic profiles: stomach content data and $\delta^{15}N$–$\delta^{13}C$

Inter- and intra-specific differences in calculated TP by size class were evident and would be expected considering size, diet, and habitat–movement characteristics of the two species. For example, the slightly higher TP, calculated by TP_{SIA} and TP_{SCA}, of small dusky relative to small scalloped hammerhead sharks, reflected the contribution of elasmobranchs to the diet of the dusky shark. Body size of sympatric elasmobranch species at birth influences TP through associated differences in gape size and manoeuvrability. These factors may enable the larger juvenile dusky sharks to exploit seasonally abundant prey resources (Hussey et al. 2009) that juvenile scalloped hammerheads cannot, resulting in resource partitioning via habitat expansion.

Large scalloped hammerhead sharks fed at a higher TP than dusky sharks based on TP_{SIA}, while TP_{SCA} of male scalloped hammerheads was similar and female TP_{SCA} was lower. Differences in the diet of small and large scalloped hammerhead sharks have been attributed to a switch in resource base driven by a move from coastal to pelagic environments (Klimley 1987). In agreement with our diet data, neritic and pelagic cephalopods have been reported as an important component of the diet of large female sharks (Clarke 1971; Klimley 1987). When considering reported $\delta^{15}N$ data for pelagic ommastrephid squids (10‰–15‰: Parry 2008; Cherel et al. 2009) and a diet discrimination factor of 2.3‰ (Hussey et al. 2010*a*), the $\delta^{15}N$ values of large female scalloped hammerhead sharks and associated TP_{SIA}, in conjunction with both

diet data and the shift in $\delta^{13}C$ (see discussion on habitat below), indicate a switch in both habitat and resource use. For large female scalloped hammerheads, TP_{SCA} was underestimated by the broad functional prey group of cephalopods, encompassing both large voracious pelagic species and small coastal species. This highlights the complexity of using broad, functional, trophic-level prey categories in the calculation of TP of large predators and suggests that published TP_{SCA} values are likely underestimated in certain cases. For large male scalloped hammerhead sharks, the importance of elasmobranchs in the diet was unusual (Klimley 1987, Stevens and Lyle 1989) but supported the observed increase in $\delta^{15}N$ and TP_{SIA}. The marginal discrepancy between TP_{SIA} of large male and female scalloped hammerhead sharks may have been a result of the elasmobranch diet component of male sharks and (or) variable $\delta^{13}C$ profiles between sexes.

The marked difference in magnitude between TP_{SCA} and TP_{SIA} for inactive, pregnant, and postpartum dusky sharks was a result of the different integrative periods of the two TP methods. Dudley et al. (2005) reported a scarcity of pregnant dusky sharks other than those at or near term in KZNSB net catches, suggesting individuals are only present in the coastal region at the point of parturition. In addition, KZN coastal waters constitute known nursery grounds for several elasmobranch species (Bass et al. 1973, 1975), and elasmobranchs commonly occur in the diet of large sharks (Cliff and Dudley 1991*a*, 1991*b*). The higher TP_{SCA} of pregnant–postpartum dusky sharks was therefore a result of the snapshot sampling of stomach content analysis (possibly 2–3 weeks) compared with the long-term integrated signature of TP_{SIA}. It is important to note that the observed low $\delta^{15}N$ values and calculated TP_{SIA} of pregnant and postpartum females may also be slightly biased by two possible factors. Corresponding $\delta^{13}C$ values of these sharks were depleted relative to reproductively inactive sharks. Based on the assumption that pelagic food webs are more depleted in $\delta^{13}C$ than coastal food webs (Hill et al. 2006), and associated shifts in $\delta^{15}N$ are reported (Sherwood and Rose 2005), these sharks may either be feeding offshore or migrating to KZN for parturition from other geographic localities (i.e., movement from isotopically distinct food webs at different latitudes). Considering we accounted for the base $\delta^{13}C$ of both inshore and offshore food webs in KZN in our two-source TP_{SIA} model, latitude effects may be the driving factor. Alternatively, the low $\delta^{15}N$ and calculated TP_{SIA} may be a result of preferential ^{15}N excretion from the mother to near-term young through the placental connection. Enriched $\delta^{15}N$ values in near-term pups relative to mothers have been observed for both carcharhinid and lamnid sharks (McMeans et al. 2009; N.E. Hussey and A.T. Fisk, unpublished data).

With the exception of large female dusky sharks, the overall ontogenetic TP_{SCA} and TP_{SIA} trends for each species were similar, although the magnitudes differed in the case of the scalloped hammerhead sharks and TP_{SCA} declined in male dusky sharks. Ontogenetic shifts in diet have been reported for several elasmobranchs (see review by Wetherbee and Cortés 2004), and a corresponding increase in $\delta^{15}N$ with size of animal has been shown for several marine taxa (e.g., Graham et al. 2007; Cherel et al. 2009; Newsome et al. 2009). This is in general agreement with our scalloped hammerhead shark data but contrasts the results in the dusky shark, where $\delta^{15}N$ and TP_{SIA} decreased with size and TP_{SCA} remained relatively constant (excluding pregnant and postpartum females). Low TP_{SCA} estimates of the large inactive male and female dusky sharks may be explained by a bias to sampling this size class during the annual sardine run, the principal period when these sharks are present in coastal waters (Dudley et al. 2005). For TP_{SIA}, the ontogenetic profile in the dusky shark is more puzzling. Compared with scalloped hammerheads, large dusky sharks are equipped with a substantially larger gape size and serrated teeth form, typically associated with predators feeding and (or) scavenging on large prey. Spear fishermen in northern KZN report frequent sightings of large dusky sharks throughout the year on or near the edge of the continental shelf (A. Heydorn, Richards Bay, KZN, personal communication, 2009). Two possibilities are therefore proposed for the observed TP_{SIA} ontogenetic trend in dusky sharks: (*i*) Small schooling fish are abundant in the region, the resulting predator–prey encounter rates are high, and energetic costs of foraging (prey chase down and manipulation) are minimal, providing large dusky sharks with an adequate prey base of lower trophic-level species. Small dusky sharks incorporate an elasmobranch component in their diet because of the abundance of elasmobranch prey in the coastal environment, and therefore overall TP values are similar between life stages. (*ii*) Large dusky sharks are incorporating larger pelagic prey in their diet on the edge of the continental shelf where system $\delta^{15}N$ values are lower, and the two-source TP model or selected base species were ineffective at accounting for this. The degree of variation between the small and large inactive dusky shark $\delta^{13}C$ signatures (more pronounced for pregnant and postpartum females) would suggest a combination of the above two possibilities, which results in dilution of the observed $\delta^{13}C$ and $\delta^{15}N$ values.

Coarse resolution size-based habitat profiles: $\delta^{13}C$

Small male scalloped hammerhead shark $\delta^{13}C$ values were highly clustered. Fennessy (1994) and de Bruyn et al. (2005) reported high catches of small scalloped hammerheads in the prawn trawl fishery on the Tugela Banks and in KZNSB beach protection nets, respectively. These observations, in conjunction with the $\delta^{13}C$ data, indicate that the home range for this size class of male scalloped hammerhead is restricted. For female scalloped hammerhead sharks of 120–160 cm PCL, $\delta^{13}C$ values were depleted relative to predicted continental shelf values, indicating prolonged residence in offshore–pelagic waters. Klimley (1987) documented that schools of scalloped hammerhead sharks on a seamount in the Gulf of Mexico consisted of predominantly female animals of 140–180 cm total length, in agreement with our data.

For male scalloped hammerhead sharks of ~80 cm PCL, $\delta^{13}C$ values followed a trend of enrichment with increasing size. Male sharks of ~140–160 cm PCL were enriched ~1‰ to predicted continental shelf values and 2.5‰ to corresponding female sharks of the same size. When considering the known $\delta^{13}C$ gradient in secondary consumers in South African waters (Hill et al. 2006), these enriched $\delta^{13}C$ values suggest that male sharks remain on the continental shelf but undertake southerly movement with a period of residency in the Eastern Cape – Transkei region. These movement and residency patterns are supported by tag–recapture data (Die-

mer et al. 2011). The high catch rate of male scalloped hammerhead sharks relative to females in KZNSB net installations further supports sexual segregation in this species (de Bruyn et al. 2005). Surprisingly, the $\delta^{13}C$ signatures of large individuals of both sexes were similar to signatures of small animals residing in nursery habitat in KZN. Our current knowledge of the ecology of large scalloped hammerheads is limited. Bass et al. (1975) reported that adult females were rare in KZN waters, while large males were common during summer. Clarke (1971), Branstetter (1987), and Stevens and Lyle (1989) reported that large female scalloped hammerheads were rare in coastal waters of Hawaii (except at the time of parturition), northwestern Gulf of Mexico, and off Northern Australia, respectively. In schools of hammerhead sharks in the Gulf of California, Klimley (1987) reported that few animals larger than 200 cm total length were present. Clarke (1971) suggested that large hammerheads may remain offshore, but our data do not confirm this. The observed $\delta^{13}C$ signatures may suggest these animals are highly migratory both by latitude and between inshore and offshore environments, incorporating $\delta^{13}C$ signatures from various isotopically distinct regions.

For dusky sharks, $\delta^{13}C$ values of small animals were more variable, with some individuals up to ~130 cm PCL enriched in $\delta^{13}C$ relative to small scalloped hammerhead sharks. These enriched $\delta^{13}C$ values may reflect the southern migration of small dusky sharks to the Eastern Cape region and a period of residency in Eastern Cape waters (Bass et al. 1973; Hussey et al. 2009). Variable $\delta^{13}C$ values may also indicate that small dusky sharks adopt different life strategies, with some "nomadic" individuals undertaking migrations, while others remain resident in KZN throughout the year (Hussey et al. 2009). The overall trend of decreasing $\delta^{13}C$ with increasing size would suggest larger animals of both sexes are spending periods of time foraging in shelf edge – offshore waters, which are typically depleted in ^{13}C (Sherwood and Rose 2005). Compagno (1984) documented that large dusky sharks occupy an intermediate offshore coastal habitat. The more depleted $\delta^{13}C$ values and larger $\delta^{13}C$ variability of pregnant and postpartum dusky sharks could be a result of a reproductive separation within the large female size class, with pregnant females spending proportionally more time in offshore waters or at higher latitudes. Alternatively, it may be indicative of the immigration of pregnant females to KZN for parturition from multiple geographic localities and (or), as previously discussed, a result of a physiological process of isotope transfer between mother and pups.

Stable isotopes as a tool to examine TP and habitat use of large sharks

Understanding both TP_{SCA} and TP_{SIA} ontogenetic profiles of large predators in the marine environment is complex. Parameters affecting calculated TP_{SCA} are documented, but the complexities associated with stable isotope trophic profiling are less well understood. Multiple factors may influence our calculations and interpretations of data, including (*i*) movement between isotopically distinct food webs (coastal–pelagic–latitude: Weng et al. 2005; Queiroz et al. 2010), (*ii*) knowledge that sharks undertake variable movement strategies (diel movement: Klimley and Nelson 1984; seasonal migrations: Domeier and Nasby-Lucas 2008; Hussey et al. 2009; large-scale movement and transoceanic migrations: Bonfil et al. 2005), (*iii*) ontogenetic–gender physiological changes (growth rate: Natanson et al. 2002), (*iv*) mother × developing young interactions (McMeans et al. 2009), and (*v*) diverse diets within a single food web (coastal (demersal–midwater), offshore (shallow pelagic – mesopelagic); Wetherbee and Cortés 2004). Accurate interpretation of stable isotope data of large mobile predators therefore requires the disentangling of the connectivity and interplay between the $\delta^{15}N$ and $\delta^{13}C$ ontogenetic profiles (Layman et al. 2007). For sharks, our lack of understanding of their movement among and residency within multiple isotopically distinct environments and the limited availability of accurate isoscapes may confound or limit the potential of two-source TP_{SIA} models. In many instances, the importance of variable $\delta^{13}C$ values on observed $\delta^{15}N$ trends and hence trophic profiles are overlooked (France et al. 1998; Parry 2008). By adopting a multifaceted approach encompassing stomach content data and $\delta^{15}N$ and $\delta^{13}C$ ontogenetic profiles, we increased the level of confidence in the conclusions drawn from the data. Future work could include sampling multiple tissues with different metabolic turnover rates to better understand diet and movement effects on ontogenetic TP profiles (MacNeil et al. 2005, 2006).

Where discrete geographical gradients in baseline $\delta^{13}C$ values occur, $\delta^{13}C$ profiles of long-term integrative muscle tissue of large predatory sharks provide accurate insights into course resolution ontogenetic movement patterns as previously described for marine mammals (e.g., Best and Schell 1996). Understanding movement and defining migration corridors of large marine vertebrates is critical for species protection and management (Weng et al. 2005). Stable isotope data of large mobile predators can therefore provide an important platform for the development of a priori hypotheses for the efficient design and implementation of species-specific satellite tagging programs and for validating population-level movement–residency patterns. It is important to note, however, that this is dependent on the availability of established baseline isotope data for the study region.

Our study indicates that sharks as top predators interact within multiple components of the marine system and raises important questions over the proportional influence exerted on single food web components by species, gender, and (or) size class effects. Furthermore, when considering community-based stable isotope approaches, if only large individuals are sampled, TP estimates for predators may be biased by associated $\delta^{13}C$ values. With the recent advancement of isoscapes (Bearhop et al. 2005), and through directed satellite tagging programs, future work will advance the development of multi-source productivity – baseline consumer models in conjunction with movement–residency time periods of large sharks to improve isotopic predictions of TP.

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References

Bass, A.J., D'Aubrey, J.D., and Kistnasamy, N. 1973. Sharks of the east coast of southern Africa. I. The genus *Carcharhinus* (Carcharhinidae). Oceanog. Res. Inst. (Durban). Investig. Rep. No. 33.

Bass, A.J., D'Aubrey, J.D., and Kistnasamy, N. 1975. Sharks of the east coast of southern Africa. III. The families Carcharhinidae (excluding *Mustelus* and *Carcharhinus*) and Sphyrnidae. Oceanog. Res. Inst. (Durban). Investig. Rep. No. 38.

Bearhop, S., Fiedler, W., Furness, R.W., Votier, S.C., Waldron, S., Newton, J., Bowen, G.J., Berthold, P., and Farnsworth, K. 2005. Assortative mating as a mechanism for rapid evolution of a migratory divide. Science, **310**(5747): 502–504. doi:10.1126/science.1115661. PMID:16239479.

Best, P.B., and Schell, D.M. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar. Biol. **124**(4): 483–494. doi:10.1007/BF00351030.

Block, B.A., Dewar, H., Blackwell, S.B., Williams, T.D., Prince, E.D., Farwell, C.J., Boustany, A., Teo, S.L.H., Seitz, A., Walli, A., and Fudge, D. 2001. Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. Science, **293**(5533): 1310–1314. doi:10.1126/science.1061197. PMID:11509729.

Bonfil, R., Meÿer, M., Scholl, M.C., Johnson, R., O'Brien, S., Oosthuzien, H., Swanson, S., Kotze, D., and Paterson, M. 2005. Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science, **310**(5745): 100–103. doi:10.1126/science.1114898. PMID:16210537.

Branstetter, S. 1987. Age, growth and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the northwestern Gulf of Mexico. Environ. Biol. Fishes, **19**(3): 161–173. doi:10.1007/BF00005346.

Cherel, Y., Fontaine, C., Jackson, G.D., Jackson, C.H., and Richard, P. 2009. Tissue, ontogenetic and sex-related differences in $\delta^{13}C$ and $\delta^{15}N$ values of the oceanic squid, *Todarodes filippovae* (Cephalopoda: Ommastrephidae). Mar. Biol. **156**(4): 699–708. doi:10.1007/s00227-008-1121-x.

Clarke, T.A. 1971. The ecology of the scalloped hammerhead shark, *Sphyrna lewini* in Hawai'i. Pac. Sci. **25**: 133–144.

Cliff, G., and Dudley, S.F.J. 1991*a*. Sharks caught in the protective gill nets off Natal, South Africa. 5. The java shark, *Carcharhinus amboinensis* (Müller and Henle). S. Afr. J. Mar. Sci. **11**(1): 443–453. doi:10.2989/025776191784287817.

Cliff, G., and Dudley, S.F.J. 1991*b*. Sharks caught in the protective gill nets off Natal, South Africa. 4. The bull shark, *Carcharhinus leucas* Valenciennes. S. Afr. J. Mar. Sci. **10**(1): 253–270. doi:10.2989/025776191091504636.

Cohen, J.E., Pimm, S.L., Yodzis, P., and Saldana, J. 1993. Body sizes of animal predators and animal prey in food webs. J. Anim. Ecol. **62**(1): 67–78. doi:10.2307/5483.

Compagno, L.J.V. 1984. FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. FAO Fish. Synop. 125, Rome, Italy.

Cortés, E. 1999. Standardised diet compositions and trophic levels of sharks. ICES J. Mar. Sci. **56**(5): 707–717. doi:10.1006/jmsc.1999.0489.

de Bruyn, P., Dudley, S.F.J., Cliff, G., and Smale, M.J. 2005. Sharks caught in the protective gill nets off KwaZulu-Natal, South Africa. 11. The scalloped hammerhead shark *Sphyrna lewini* (Griffith and Smith). Afr. J. Mar. Sci. **27**(3): 517–528. doi:10.2989/18142320509504112.

Diemer, K.M., Mann, B.Q., and Hussey, N.E. 2011. Distribution and movements of scalloped hammerhead *Sphyrna lewini* and smooth hammerhead *Sphyrna zygaena* sharks along the east coast of southern Africa. Afr. J. Mar. Sci. **33**(2): 229–238.

Domeier, M.L., and Nasby-Lucas, N. 2008. Migration patterns of white sharks *Carcharodon carcharias* tagged at Guadalupe Island, Mexico, and identification of an eastern Pacific shared offshore foraging area. Mar. Ecol. Prog. Ser. **370**: 221–237. doi:10.3354/meps07628.

Dudley, S.F.J., Cliff, G., Zungu, M.P., and Smale, M.J. 2005. Sharks caught in the protective gill nets of KwaZulu-Natal, South Africa. 10. The dusky shark *Carcharhinus obscurus* (Lesuer 1818). Afr. J. Mar. Sci. **27**(1): 107–127. doi:10.2989/18142320509504072.

Dulvy, N.K., Polunin, N.V.C., Mill, A.C., and Graham, N.A.J. 2004. Size structural change in lightly exploited coral reef fish communities: evidence for weak indirect effects. Can. J. Fish. Aquat. Sci. **61**(3): 466–475. doi:10.1139/f03-169.

Elton, C.S. 1927. Animal ecology. Sidgwick and Jackson, London, UK.

Estrada, J.A., Rice, A.N., Lutcavage, M.E., and Skomal, G.B. 2003. Predicting trophic position of sharks of the north-west Atlantic Ocean using stable isotope analysis. J. Mar. Biol. Assoc. U.K. **83**(6): 1347–1350. doi:10.1017/S0025315403008798.

Estrada, J.A., Rice, A.N., Natanson, L.J., and Skomal, G.B. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology, **87**(4): 829–834. doi:10.1890/0012-9658(2006)87[829:UOIAOV]2.0.CO;2. PMID:16676526.

Fennessy, S.T. 1994. Incidental capture of elasmobranchs by commercial prawn trawlers on the Tugela banks, Natal, South Africa. S. Afr. J. Mar. Sci. **14**(1): 287–296. doi:10.2989/025776194784287094.

Fisk, A.T., Tittlemier, J.L., Pranschke, J.L., and Norstrom, R.J. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of the Greenland shark. Ecology, **83**(8): 2162–2172. doi:10.1890/0012-9658(2002)083[2162:UACASI]2.0.CO;2.

France, R., Chandler, M., and Peters, R. 1998. Mapping trophic continua of benthic food webs: body size – $\delta^{15}N$ relationships. Mar. Ecol. Prog. Ser. **174**: 301–306. doi:10.3354/meps174301.

Graham, B.S., Grubbs, D., Holland, K.N., and Popp, B.N. 2007. A rapid ontogenetic shift in the diet of juvenile yellowfin tuna from Hawaii. Mar. Biol. **150**(4): 647–658. doi:10.1007/s00227-006-0360-y.

Hill, J.M., and McQuaid, C.D. 2008. $\delta^{13}C$ and $\delta^{15}N$ biogeographic trends in rocky intertidal communities along the coast of South Africa: evidence of strong environmental signatures. Estuar. Coast. Shelf Sci. **80**(2): 261–268. doi:10.1016/j.ecss.2008.08.005.

Hill, J.M., McQuaid, C.D., and Kaehler, S. 2006. Biogeographic and nearshore–offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. Mar. Ecol. Prog. Ser. **318**: 63–73. doi:10.3354/meps318063.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia, **120**(3): 314–326. doi:10.1007/s004420050865.

Hussey, N.E., McCarthy, I.D., Dudley, S.F.J., and Mann, B. 2009. Nursery grounds, movement patterns and growth rates of dusky sharks (*Carcharhinus obscurus*): a long-term tag and release study in South African waters. Mar. Freshw. Res. **60**(6): 571–583. doi:10.1071/MF08280.

Hussey, N.E., Brush, J., and Fish, A.T. 2010*a*. $\delta^{15}N$ and $\delta^{13}C$ diet discrimination factors for large sharks under controlled conditions. Comp. Biochem. Physiol. B, **155**: 445–453.

Hussey, N.E., MacNeil, M.A., and Fisk, A.T. 2010*b*. The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. Hydrobiologia, **654**(1): 1–5. doi:10.1007/s10750-010-0361-1.

Hussey, N.E., McCann, H., Cliff, G., Dudley, S.F.J., Wintner, S.P., and Fisk, A.T. 2011. Size-based analysis of the diet and trophic position of the white shark (*Carcharodon carcharias*) in South African waters. *In* Global perspectives on the biology and life history of the great white shark. *Edited by* M.L. Domeier. CRC Press, Boca Raton, Fla., USA. [In press.]

Hyslop, E.J. 1980. Stomach content analysis: a review of methods and their application. J. Fish Biol. **17**(4): 411–429. doi:10.1111/j.1095-8649.1980.tb02775.x.

Jennings, S., Pinnegar, J.K., Polunin, N.V.C., and Boon, T.W. 2001. Weak cross-species relationships between body size and trophic level belie powerful size-based trophic structuring in fish communities. J. Anim. Ecol. **70**(6): 934–944. doi:10.1046/j.0021-8790.2001.00552.x.

Klimley, A.P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. Environ. Biol. Fishes, **18**(1): 27–40. doi:10.1007/BF00002325.

Klimley, A.P., and Nelson, D.R. 1984. Diel movement patterns of the scalloped hammerhead shark (*Sphyrna lewini*) in relation to El Bajo Espiritu Santo: a refuging central-position social system. Behav. Ecol. Sociobiol. **15**(1): 45–54. doi:10.1007/BF00310214.

Layman, C.A., Winemiller, K.O., Arrington, D.A., and Jepsen, D.B. 2005. Body size and trophic position in a diverse tropical food web. Ecology, **86**(9): 2530–2535. doi:10.1890/04-1098.

Layman, C.A., Arrington, D.A., Montana, C.G., and Post, D.M. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology, **88**(1): 42–48. doi:10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2. PMID:17489452.

MacNeil, M.A., Skomal, G.B., and Fisk, A.T. 2005. Stable isotopes from multiple tissues reveal diet switching in sharks. Mar. Ecol. Prog. Ser. **302**: 199–206. doi:10.3354/meps302199.

MacNeil, M.A., Drouillard, K.G., and Fisk, A.T. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can. J. Fish. Aquat. Sci. **63**(2): 345–353. doi:10.1139/f05-219.

McMeans, B.C., Olin, J.A., and Benz, G.W. 2009. Stable isotope comparisons between embryos and mothers of a placentatrophic shark species. J. Fish Biol. **75**(10): 2464–2474. doi:10.1111/j.1095-8649.2009.02402.x. PMID:20738502.

Mucientes, G.R., Queiroz, N., Sousa, L.L., Tarroso, P., and Sims, D.W. 2009. Sexual segregation of pelagic sharks and the potential threat from fisheries. Biol. Lett. **5**(2): 156–159. doi:10.1098/rsbl.2008.0761. PMID:19324655.

Myers, R.A., and Worm, B. 2003. Rapid worldwide depletion of predatory fish communities. Nature, **423**(6937): 280–283. doi:10.1038/nature01610. PMID:12748640.

Myers, R.A., Baum, J.K., Shepherd, T.D., Powers, S.P., and Peterson, C.H. 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science, **315**(5820): 1846–1850. doi:10.1126/science.1138657. PMID:17395829.

Natanson, L.J., Mello, J.J., and Campana, S.E. 2002. Validated growth and age of the porbeagle shark (*Lamna nasus*) in the western North Atlantic Ocean. Fish Bull. **100**: 266–278.

Newsome, S.D., Etnier, M.A., Monson, D.H., and Fogel, M.L. 2009. Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Mar. Ecol. Prog. Ser. **374**: 229–242. doi:10.3354/meps07747.

Olin, J.A., Hussey, N.E., Fritts, M., Heupel, M.R., Simpfendorfer, C.A., Poulakis, G.R., and Fisk, A.T. 2011. Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. Rapid Commun. Mass Spectrom. **25**(8): 1008–1016. doi:10.1002/rcm.4946. PMID:21452377.

Parry, M. 2008. Trophic variation with length in two ommastrephid squids, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*. Mar. Biol. **153**(3): 249–256. doi:10.1007/s00227-007-0800-3.

Pauly, D., Christensen, J., V, Dalsgaard, J., Froese, R., and Torres, F., *Jr.* 1998. Fishing down marine food webs. Science, **279**(5352): 860–863. doi:10.1126/science.279.5352.860. PMID:9452385.

Pinnegar, J.K., Jennings, S., O'Brien, C.M., and Polunin, N.V.C. 2002. Long-term changes in the trophic level of the Celtic Sea fish community and fish market price distribution. J. Appl. Ecol. **39**(3): 377–390. doi:10.1046/j.1365-2664.2002.00723.x.

Post, D.M. 2002. Using stable isotope to estimate trophic position: models, methods and assumptions. Ecology, **83**(3): 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.

Queiroz, N., Humphries, N.E., Noble, L.R., Santos, A.M., and Sims, D.W. 2010. Short-term movements and diving behaviour of satellite tracked blue shark (*Prionace glauca*), in the north-eastern Atlantic ocean. Mar. Ecol. Prog. Ser. **406**: 265–279. doi:10.3354/meps08500.

Rubenstein, D.R., and Hobson, K.A. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol. Evol. **19**(5): 256–263. doi:10.1016/j.tree.2004.03.017. PMID:16701265.

Sherwood, G.D., and Rose, G.A. 2005. Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. Estuar. Coast. Shelf Sci. **63**(4): 537–549. doi:10.1016/j.ecss.2004.12.010.

Stevens, J.D., and Lyle, J.M. 1989. Biology of three hammerhead sharks (*Eusphyra blochii*, *Sphyrna mokarran* and *S. lewini*) from Northern Australia. Aust. J. Mar. Freshwater Res. **40**(2): 129–146. doi:10.1071/MF9890129.

Vander Zanden, M.J., Cabana, G., and Rasmussen, J.B. 1997. Comparing the trophic position of freshwater fish calculated using stable nitrogen isotope ratios $\delta^{15}N$ and literature dietary data. Can. J. Fish. Aquat. Sci. **54**(5): 1142–1158. doi:10.1139/f97-016.

Weng, K.C., Castilho, P.C., Morrissette, J.M., Landeira-Fernandez, A.M., Holts, D.B., Schallert, R.J., Goldman, K.J., and Block, B.A. 2005. Satellite tagging and cardiac physiology reveal niche expansion in salmon sharks. Science, **310**(5745): 104–106. doi:10.1126/science.1114616. PMID:16210538.

Wetherbee, B.M., and Cortés, E. 2004. Food consumption and feeding habits. *In* Biology of sharks and their relatives. *Edited by* J.C. Carrier, J.A. Musick, and M.R. Heithaus. CRC Press, Boca Raton, Fla. pp. 225–246.

White, W.T., Platell, M.E., and Potter, I.C. 2004. Comparisons between the diets of four abundant species of elasmobranchs in a subtropical embayment: implications for resource partitioning. Mar. Biol. **144**(3): 439–448. doi:10.1007/s00227-003-1218-1.

Appendix A

Tables A1 and A2 appear on the following pages.

Table A1. The diet of the scalloped hammerhead shark (*Sphyrna lewini*) off KwaZulu-Natal, South Africa, from archived stomach content data (1983–2006).

	Small				Medium				Large			
	% Mass		% IRI		% Mass		% IRI		% Mass		% IRI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Teleost	**62.98**	**67.37**	**55.63**	**83.87**	**52.35**	**56.28**	**54.80**	**57.56**	**49.24**	**49.76**	**64.21**	**68.37**
Acanthuridae	0.56		0.03									
Anguillidae	4.37	2.17	0.33	0.21	1.82	0.24	0.29	0.01		0.90		0.21
Ariidae	0.72	0.77	0.07	0.04	0.49	2.25	0.15	0.33		1.91		0.53
Balistidae												
Belonidae						0.07		0.00				
Carangidae	0.73	1.88	0.07	0.34		0.08		0.04		0.05		0.01
Cheilodactyleidae					3.57	0.58	0.22	0.01				
Chirocentridae												
Chlorophthalmidae												
Cichlidae												
Clupeidae	1.58	0.24	0.24	0.01		1.22		0.16		1.37		0.37
Congridae	0.26		0.01									
Cynoglossidae												
Dactylopteridae									0.75		0.15	
Elopidae												
Engraulidae		0.09		0.00						0.04		0.03
Ephippidae												
Exocetidae		1.57		0.06		0.32		0.01				
Exocoetidae												
Gerreidae												
Haemulidae	0.39	3.75	0.04	2.95	2.54	1.31	1.40	1.30		15.81		13.66
Istiophoridae												
Leiognathidae	0.06	1.30	0.02	0.15		0.26		0.01		0.06		0.01
Mugilidae						0.22		0.01		0.15		0.02
Mullidae	2.89	1.39	0.12	0.02		1.05		0.02				
Muraenidae												
Mycteophidae					0.06		0.03					
Oplegnathidae						0.14		0.00		1.74		0.63
Ostraciidae	0.52		0.02			0.21		0.01				
Ostraciidae		0.09		0.00								
Peristediidae									1.94		0.26	
Platycephalidae		0.14		0.01		2.36		0.08				
Pleuronectidae												
Plotosidae	1.36	1.05	0.13	0.17		3.70		0.52		4.46		0.65
Pomacentridae	0.19		0.03									
Pomatomidae												
Priacanthidae		1.28		0.02								
Sciaenidae	2.31	5.19	0.43	0.77	1.85	8.52	0.30	6.23		1.81		1.89
Scombridae	6.22	4.13	0.87	0.45	10.66	1.70	3.16	0.37		8.36		1.13
Scorpididae												
Serranidae		0.91		0.07		0.18		0.00		0.35		0.03
Sparidae	3.21	12.16	1.59	8.89	4.16	9.56	1.84	10.49	7.50	0.81	2.38	1.63
Sphyraenidae		0.84		0.09	0.42	0.10	0.14	0.01	0.50		0.21	
Syngnathidae		0.04		0.00		0.01		0.00				
Synodontidae	13.37	2.71	3.31	0.16	0.49	0.20	0.15	0.02	0.60		0.14	
Terapontidae	3.37	4.57	0.42	0.73	3.86	2.11	1.31	0.53	6.38	0.13	0.68	0.02
Trichiuridae	4.40	4.10	0.31	0.65	4.69	8.11	2.58	5.91		0.79		0.16
Triglidae	0.04		0.01									
Unidentified teleost	16.43	17.03	47.58	68.08	17.74	11.77	43.23	31.47	31.56	11.01	60.38	47.39
Elasmobranch	**6.05**	**6.52**	**1.28**	**0.58**	**7.56**	**16.01**	**1.34**	**5.43**	**0.00**	**37.96**	**0.00**	**9.74**
Carcharhinidae										1.25		0.14
Dasyatidae						1.27		0.02		2.56		0.24
Gymnuridae						2.76		0.09		2.25		0.22
Lamnidae												
Mobulidae												
Myliobatidae												
Odontaspididae												
Rajidae	1.88		0.14		3.02		0.65					
Rhinobatidae	0.44	2.76	0.02	0.11	1.63	5.34	0.18	2.12		11.54		1.42
Rhinopteridae												

Table A1 (*concluded*).

	Small				Medium				Large			
	% Mass		% IRI		% Mass		% IRI		% Mass		% IRI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Scyliorhinidae	3.51	2.39	1.09	0.28	1.11	6.64	0.12	3.19		8.01		3.59
Sphyrnidae												
Squalidae					1.80		0.39					
Squatinidae										4.80		0.19
Unidentified batoid		0.29		0.01								
Unidentified shark										6.96		2.98
Unidentified elasmobranch	0.21	1.07	0.03	0.17						0.58		0.95
Cephalopod	**18.87**	**24.34**	**29.00**	**15.23**	**39.07**	**23.71**	**43.53**	**33.83**	**50.69**	**11.72**	**35.62**	**21.82**
Coleoidae	1.79	1.65	0.18	0.38		0.37		0.08				
Enoploteuthidae					1.41	1.24	0.15	0.20	31.56	0.09	3.35	0.03
Loligo spp.												
Lycoteuthidae		0.18		0.01								
Octopodidae	10.40	7.59	25.03	7.81	2.93	4.69	1.58	3.72	4.89	0.43	6.10	0.08
Octopoteuthidae	0.41	4.63	0.10	1.13	16.44	0.20	3.55	0.03	13.22	9.71	25.91	12.83
Ommastrephidae												
Sepiidae (cuttlefish)	6.28	10.20	3.70	5.90	2.11	3.09	2.64	3.58	1.02	1.50	0.26	8.88
Teuthoidea (squids)						13.90		26.22				
Unidentified cephalopod		0.07		0.00	16.17	0.22	35.60	0.01				
Crustacean	**12.10**	**1.78**	**14.09**	**0.32**	**0.99**	**4.01**	**0.23**	**3.17**	**0.06**	**0.00**	**0.09**	**0.00**
Anomura (hermit crab)												
Brachyura (crabs)	0.11		0.03		0.09	2.05	0.02	2.55				
Macrura (rock lobsters)		1.17		0.22	0.48	0.04	0.10	0.01	0.06		0.09	
Mantis shrimp	11.90		14.06			1.56		0.51				
Unidentified prawn		0.37		0.09	0.36	0.27	0.12	0.08				
Unidentified crustacean	0.09	0.21	0.01	0.01	0.06	0.09	0.01	0.02				
Mammal	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Tursiops aduncus												
Unidentified dolphin												
Unidentified cetacean												
Unidentified mammal												
Unidentified Mysticeti												
Unidentified seal												
Bird	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.08**	**0.00**
Unidentified bird						0.00		0.00	0.01		0.08	
Miscellaneous	**0.00**	**0.03**	**0.00**	**0.00**	**0.04**	**0.00**	**0.07**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Bivalve					0.04		0.07					
Gastropod		0.03		0.00								
Fisherman's bait												

Note: % Mass, percent gravimetric mass; % IRI, percent index of relative importance. For sample sizes, see Fig. 2.

Table A2. The diet of the dusky shark (*Carcharhinus obscurus*) off KwaZulu-Natal, South Africa, from archived stomach content data (1983–2006).

	Small				Medium				Large			
	% Mass		% IRI		% Mass		% IRI		% Mass		% IRI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Teleost	**65.67**	**54.27**	**91.80**	**82.46**	**31.98**	**43.28**	**64.64**	**67.93**	**12.06**	**78.95**	**78.13**	**99.06**
Acanthuridae												
Anguillidae	1.44		0.12		0.21		0.01					
Ariidae					0.19		0.02					
Balistidae	0.16		0.02		0.35	0.09	0.01	0.00				
Belonidae												
Carangidae	0.31	0.97	0.16	0.11	2.16	0.92	0.17	0.05	0.01		0.00	
Cheilodactylyeidae					0.34	0.24	0.02	0.01				
Chirocentridae												
Chlorophthalmidae	0.39		0.02									
Cichlidae					0.22	0.15	0.01	0.00				
Clupeidae	2.23	1.99	0.17	1.01	11.88	32.84	36.10	60.67	10.39	78.74	77.08	99.03
Congridae												
Cynoglossidae	0.04		0.01									
Dactylopteridae												
Elopidae												
Engraulidae	0.03		0.01		1.57	0.06	5.25	0.01				
Ephippidae					1.25		0.03					
Exocetidae												
Exocoetidae						0.59		0.02				
Gempylidae		1.67		0.06								
Gerreidae	0.97		0.05									
Haemulidae	0.41	0.44	0.35	0.63	0.24	0.24	0.13	0.03	0.57		0.02	
Istiophoridae									0.23		0.01	
Leiognathidae	3.97		0.11			0.14		0.01				
Mugilidae	1.55	1.11	0.12	0.46		0.36		0.01				
Mullidae						0.04		0.00				
Muraenidae		0.91		0.04	2.16							
Myctophidae												
Oplegnathidae					0.55	0.04	0.01	0.00				
Ostraciidae					0.02		0.00					
Ostraciidae												
Peristediidae												
Platycephalidae												
Pleuronectidae		0.27		0.02		0.05		0.01				
Plotosidae		1.54		0.14					0.21		0.01	
Pomacentridae												
Pomatomidae	2.28	0.73	0.31	0.10	0.11		0.00		0.11		0.00	
Priacanthidae												
Sciaenidae	1.15	7.53	0.19	1.01	0.49	0.13	0.10	0.00	0.17		0.02	
Scombridae	18.40	11.08	5.58	1.79	4.22	3.91	1.75	0.86	0.02	0.09	0.00	0.00
Scorpididae	0.11		0.01									
Serranidae												
Sparidae	5.74	1.17	3.21	0.15	0.32	0.06	0.13	0.00	0.14		0.02	
Sphyraenidae					0.08		0.00					
Sygnathidae												
Synodontidae		0.08		0.02								
Tetraodontidae												
Trichiuridae					0.01		0.00					
Triglidae												
Unidentified teleost	26.46	24.79	81.37	76.93	5.63	3.40	20.90	6.22	0.21	0.11	0.97	0.03
Elasmobranch	**21.11**	**36.14**	**2.19**	**14.79**	**65.26**	**48.97**	**32.65**	**24.35**	**78.25**	**14.26**	**20.92**	**0.85**
Carcharhinidae	1.20	1.66	0.04	0.06	17.74	4.84	8.34	0.51	29.91	8.12	9.50	0.55
Dasyatidae	4.70	11.04	0.86	3.77	10.03	3.38	4.79	0.55	0.85	0.23	0.05	0.00
Gymnuridae						0.86		0.02				
Lamnidae									5.58		0.29	
Mobulidae	0.68		0.08		0.85		0.05					
Myliobatidae					1.85		0.15		1.75		0.05	
Odontaspididae									6.76		0.54	
Rajidae									0.00		0.00	
Rhinobatidae		0.80		0.04	1.77		0.05		0.23		0.01	
Rhinopteridae	2.89		0.08						0.39		0.01	

Table A2 (*concluded*).

	Small				Medium				Large			
	% Mass		% IRI		% Mass		% IRI		% Mass		% IRI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Scyliorhinidae					0.14		0.01		6.62		0.17	
Sphyrnidae	5.27	2.86	0.30	0.21	16.96	6.51	4.35	0.50	7.18	0.52	0.96	0.01
Squalidae												
Squatinidae					0.76	0.73	0.02	0.02	0.00		0.00	
Unidentified batoid	0.62	0.24	0.08	0.02	0.08	1.71	0.01	0.04				
Unidentified elasmobranch	0.01	0.90	0.01	0.14	1.22	0.30	0.21	0.03	0.01		0.00	
Unidentified shark	5.74	18.65	0.74	10.56	13.86	30.64	14.69	22.66	18.98	5.39	9.34	0.29
Cephalopod	**5.78**	**4.68**	**1.99**	**1.97**	**2.29**	**2.37**	**1.76**	**0.84**	**0.47**	**0.01**	**0.17**	**0.00**
Coleoidae												
Enoploteuthidae												
Loligo spp.	0.05	0.01	0.01	0.02								
Lycoteuthidae												
Octopodidae	0.41	1.82	0.09	0.28	1.52	1.49	0.72	0.27	0.42		0.03	
Octopoteuthidae												
Ommastrephidae	0.02		0.01									
Sepiidae (cuttlefish)	1.71	1.32	0.68	1.26	0.65	0.62	1.01	0.55	0.05	0.01	0.14	0.00
Teuthoidea (squids)	0.41	0.01	0.23	0.02	0.09	0.09	0.03	0.00				
Unidentified cephalopod	3.17	1.52	0.96	0.40	0.02	0.17	0.00	0.02				
Crustacean	**0.43**	**0.68**	**0.13**	**0.06**	**1.47**	**3.57**	**0.87**	**6.68**	**0.01**	**0.00**	**0.01**	**0.00**
Anomura (hermit crab)												
Brachyura (crabs)	0.07		0.05		0.63	1.10	0.35	0.48	0.01		0.01	
Macrura (rock lobsters)	0.12	0.60	0.05	0.03	0.08	0.18	0.03	0.04				
Mantis shrimp					0.75	2.29	0.49	6.16				
Unidentified prawn	0.17	0.06	0.02	0.02	0.02		0.00					
Unidentified crustacean	0.07	0.02	0.01	0.02	0.00	0.00	0.00	0.00				
Mammal	**1.33**	**3.73**	**0.11**	**0.42**	**1.12**	**1.82**	**0.06**	**0.20**	**9.20**	**2.98**	**0.77**	**0.04**
Tursiops aduncus									3.27	2.98	0.17	0.04
Unidentified dolphin	1.33	3.73	0.11	0.42	0.46	1.82	0.03	0.20	0.27		0.01	
Unidentified Mysticeti									5.36		0.58	
Unidentified seal									0.30		0.01	
Unidentified cetacean												
Unidentified mammal					0.65		0.04					
Bird	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Unidentified bird												
Miscellaneous	**5.69**	**0.50**	**3.78**	**0.30**	**0.04**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Bivalve												
Gastropod					0.01		0.01					
Fisherman's bait	5.69	0.50	3.78	0.30	0.03		0.00					

Note: % Mass, percent gravimetric mass; % IRI, percent index of relative importance. For sample sizes, see Fig. 2.

Regional patterns in mercury and selenium concentrations of yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*) in the Pacific Ocean

Bridget E. Ferriss and Timothy E. Essington

Abstract: Mercury (Hg) concentrations in high trophic level fish, such as bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*), can often exceed consumption advisories. Here we sampled 444 yellowfin and bigeye tuna to determine whether tuna Hg concentration varies regionally in the eastern and central Pacific Ocean and whether this variation corresponds to environmental characteristics that promote the bioavailability of Hg. Of the five regions sampled, we found significantly higher Hg concentrations in the eastern equatorial region (5°S–5°N; 110°W–120°W) for both species. Hg concentrations in this region were elevated by 0.22 and 0.17 $\mu g \cdot g^{-1}$ for yellowfin and bigeye tuna, respectively, compared with Hg concentrations in the other regions. Tuna selenium concentrations, which may alter the toxicity of Hg, did not vary by region. Oceanographic data indicated that the eastern equatorial region had elevated chlorophyll *a* concentrations and shallow minimum oxygen depths, both of which promote Hg methylation. These findings suggest that methylation-promoting mechanisms may translate into regional variation in the Hg concentrations of highly mobile, high trophic level fish.

Résumé : Les concentrations de mercure (Hg) dans les poissons de haut niveau trophique, tels que le thon ventru (*Thunnus obesus*) et l'albacore à nageoires jaunes (*Thunnus albacares*), peuvent souvent dépasser les niveaux recommandés pour la consommation. Nous avons échantillonné 444 thons et albacores pour voir si les concentrations de Hg varient en fonction de la région dans l'est et le centre du Pacifique et si cette variation correspond aux caractéristiques environnementales qui favorisent la biodisponibilité du Hg. Parmi les cinq régions échantillonnées, il existe des concentrations significativement plus élevées de Hg dans la région équatoriale orientale (5°S–5°N; 110°W–120°W) chez les deux espèces. Par comparaison aux autres régions, les concentrations de Hg y sont plus élevées respectivement de 0,22 et de 0,17 $\mu g \cdot g^{-1}$ chez le thon et l'albacore. Les concentrations de sélénium chez les thons et les albacores, qui peuvent modifier la toxicité du Hg, ne varient pas selon les régions. Les données océanographiques indiquent que la région équatoriale orientale possède des concentrations élevées de chlorophylle a et des profondeurs faibles du minimum d'oxygène, deux facteurs qui favorisent la méthylation du Hg. Nos résultats indiquent que les mécanismes qui favorisent la méthylation peuvent se refléter en une variation régionale des concentrations de Hg chez les poissons de haut niveau trophique et à forte mobilité.

[Traduit par la Rédaction]

Introduction

Mercury (Hg) contamination of food sources is a global health concern owing to its neurotoxic properties. Humans are primarily exposed to Hg through consumption of fish that have elevated Hg concentrations. There is a growing recognition of the need to balance the public's awareness of fish as an important source of nutrition with their exposure to contaminants, such as methylmercury (MeHg), dioxins, and polychlorinated biphenyls (PCBs) and the associated danger to human health (Nesheim and Yaktine 2006). In many nations this is achieved by setting consumption advisories that identify species-specific limits on weekly or monthly consumption based on mean fish Hg concentrations (Nesheim and Yaktine 2006). Yet, variation in Hg within species can be substantial and exceed variation between species (Levinton and Pochron 2008). For example, large marine apex predators such as tunas (*Thunnus*) are common targets of consumption advisories but exhibit wide ranges of Hg concentrations within species (e.g., Kaneko and Ralston 2007). Moreover, the reference doses upon which consumption advisories are based do not account for potential synergistic or antagonistic interactions with other elements, such as selenium (Ganther et al. 1972).

Oceanographic conditions that promote Hg methylation and uptake at the base of the food chain may dictate total Hg concentrations in high trophic level fishes, of which approximately 95% is MeHg (Bloom 1989). In marine ecosys-

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tems, MeHg concentrations are greatest in waters with low dissolved oxygen (DO) concentrations, where sulfate-reducing microbial production of MeHg is believed to occur (Fitzgerald et al. 2007). High rates of primary productivity have also been linked to higher MeHg concentrations in the water column, potentially via increases in dissolved organic matter concentrations and particle scavenging, which acts to transport Hg to the lower oxygen depths where methylation occurs (Fitzgerald et al. 2007; but see Essington and Houser 2003). These relationships have been supported by direct measurements of MeHg in the water column that show higher concentrations in the eastern equatorial Pacific compared with those in other equatorial and tropical Pacific regions (Mason and Fitzgerald 1993).

The Pacific Ocean is defined by major surface currents, tradewinds, and physical features that create separate environments spanning a broad range of productivity and dissolved oxygen concentrations. The eastern tropical Pacific (20°S–20°N and 150°W to continental shelf; Hinke et al. 2004) is characterized by strong upwelling conditions, creating well-mixed surface waters with cooler temperatures, higher concentrations of nutrients, lower DO concentrations, and a deeper thermocline (Palacios et al. 2006). Moving westward, the cold tongue of nutrient-rich surface waters transitions to the warm pool of nutrient-poor, high-oxygen waters, definitive of the western Pacific (Mann and Lazier 1991). The central north Pacific (0°N–40°N and 130°E–150°W; Hinke et al. 2004) is characterized by low-nutrient oligotrophic waters with higher DO and mesoscale variability in the form of seasonal eddies and fronts that support local regions of productivity (Seki et al. 2002). The range of productivity and DO, among other oceanographic characteristics, found in the eastern and central equatorial and north Pacific regions could theoretically support regional differences in Hg methylation in the water column.

Though rates of methylation and availability of MeHg vary regionally, these differences may not translate into differences in Hg concentrations in upper trophic level predators. Nearly all Hg in tunas is derived from their food (Morel et al. 1998), and tuna in the eastern Pacific Ocean are known to feed on prey assemblages that are taxonomically distinct from those in the central or western Pacific (Watanabe 1960; Olson and Boggs 1986; Allain 2004). These different prey species accumulate varying concentrations of Hg (e.g., Choy et al. 2009) because of life history traits and could therefore mask or amplify any regional differences due to Hg biogeochemistry. Moreover, because tunas disperse over large distances, tuna Hg concentrations may integrate conditions experienced over large areas and may not reflect the availability of MeHg at the base of the food web at the location of capture.

The gap in knowledge of how regional differences in Hg methylation rates translate to Hg concentrations through the food web is particularly relevant for large, mobile, commercially important pelagic fish such as yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*). These species have relatively high Hg concentrations and are therefore the subject of consumption regulations. Like many other teleost apex predators, these tunas have relatively wide population ranges that span regions with both exceptionally low and high methylation rates. Their habitat extends from the productive low DO conditions in the eastern Pacific to the less productive, higher DO waters of the western Pacific (Mann and Lazier 1991). Although populations can be widely distributed, ranges of individual tuna are restricted, with a mean range of approximately 1000–2000 km (Sibert and Hampton 2003; Schaefer et al. 2007). Thus, assuming a MeHg half-life on the order of months to years (Trudel and Rasmussen 1997), individual tunas may reside in regions with distinct concentrations of Hg for sufficient periods of time for the regional differences in Hg biogeochemistry to be manifest in their tissue concentrations.

A further complication in considering the efficacy of consumption advisories in achieving the optimal trade-off between health hazards and nutritive benefits of seafood consumption derives from the potential neutralizing effect of selenium (Se) on the toxicity of Hg (Ganther and Sunde 2007). For this reason, some have argued that consumption advisories should be based on Se:Hg ratios (Kaneko and Ralston 2007). Yet, the ranges of Se concentrations and corresponding Hg:Se ratios in marine fish species in general, and pelagic tunas in particular, are not well known (but see Kaneko and Ralston 2007). Specifically, it is not known whether regional patterns of Se might be independent of or covary with patterns of Hg concentrations in these fishes.

In this study we tested the hypothesis that Hg concentrations in yellowfin and bigeye tuna vary regionally across the eastern Pacific Ocean, reflecting variation in oceanographic conditions that affect rates of Hg methylation. Specifically, we predicted that regions with characteristics previously shown to be associated with high concentrations of MeHg in the water column will coincide with high concentrations of Hg in tuna. To test this hypothesis, we sampled tuna Hg concentrations in five regions of the Pacific that provided contrasts in productivity and DO concentrations. Moreover, we measured Se concentrations in these tunas to determine the regional patterning of Se:Hg ratios.

Materials and methods

We selected five sampling regions within the central and eastern Pacific based on differences in oceanographic characteristics and separation distance relative to the potential movement of tuna (Fig. 1). Selection of sampling regions was informed by long-term measurements of oxygen and chlorophyll *a* concentrations (Table 1) available from the World Ocean Database (Boyer et al. 2006). We quantified the regional concentration of DO by calculating the depth at which DO is <2 mg·L^{-1}, also known as the threshold of hypoxia (Kamykowski and Zentara 1990), as the depth at which low DO waters occur could influence the bioavailability of Hg at the base of the food chain. Three of the regions are located along the equator, from the upwelling, productive waters in the eastern Pacific to the productive but more stratified central Pacific: the eastern equatorial Pacific (5°S–5°N; 110°W–120°W), the mid-equatorial Pacific (5°S–5°N; 130°W–140°W), and the central equatorial Pacific (5°S–5°N; 145°W–165°W). Two additional regions were located north of the equator to provide further contrasts in oceanographic conditions. The main Hawaiian Islands region (15°N–26°N; 152°W–167°W) is characterized by higher DO and lower chlorophyll, while the eastern north Pacific region (10°N–20°N; 110°W–120°W) has lower DO and lower chlorophyll.

Fig. 1. Capture locations (small open circles) and regions (large open circle and rectangles) of yellowfin and bigeye tuna analyzed in this study. The large circle around the Hawaiian Islands represents the combined area of catch locations, as obtained from longline vessel captains and logbook information. Each region is characterized by the depth (m) at which dissolved oxygen (DO) is <2 mg·L^{-1} and the chlorophyll *a* (Chl) concentration integrated over the top 100 m.

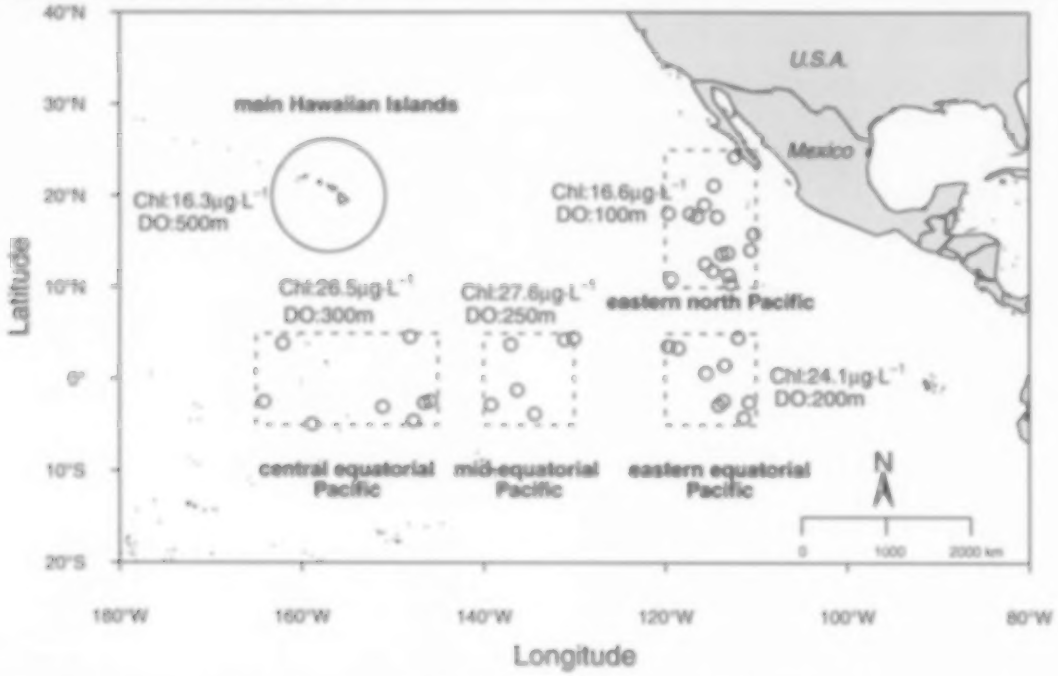


Table 1. Summary of yellowfin tuna (YFT) and bigeye tuna (BET) samples used in this study, and the mean annual chlorophyll *a* and dissolved oxygen characteristics of each region.

Region	Species	No. samples	Mass (kg)	Chlorophyll *a* (µg·L^{-1}) in top 100 m	Depth (m) at which DO is <2 mg·L^{-1}
Eastern equatorial Pacific	BET	50	3.7 (1.5)	24.1	200
	YFT	45	3.2 (0.7)		
Mid-equatorial Pacific	BET	50	4.8 (1.9)	27.6	250
	YFT	50	2.5 (1.0)		
Central equatorial Pacific	BET	50	4.5 (2.0)	26.5	300
	YFT	50	5.7 (10.2)		
Eastern north Pacific	BET	0	na	16.6	100
	YFT	49	3.3 (0.6)		
Main Hawaiian Islands	BET	50	17.0 (2.8)	16.3	500
	YFT	50	15.1 (6.8)		

Note: All tuna were caught by purse seine (2002–2003) except for those in the main Hawaiian Islands, which were caught by longline (November–December 2007). Mass data are means with SD in parentheses. Chlorophyll *a* values are integrated across the top 100 m in depth. Depths of dissolved oxygen (DO) are to the nearest 50 m. Oceanographic data are from the World Ocean Database.

Most regions were separated by at least 10° latitude and longitude, or roughly 1000 km longitude (near the equator), but two pairs of areas were separated by only 5° (the central and mid-equatorial Pacific regions and the eastern equatorial and eastern north Pacific regions) because of the lack of sufficient samples to allow regional comparisons across larger distances.

Tuna tissue samples were obtained during two sampling events (Table 1). The majority of samples were collected by fishery observers from commercial purse seine fishing vessels in the eastern Pacific Ocean (2002–2003). These samples were stored at approximately –20 °C at the Inter-American Tropical Tuna Commission. We subsampled this collection for analysis of total Hg and Se concentrations, choosing 50 individuals from each species in each of four regions in the eastern and equatorial Pacific Ocean. Because MeHg accounts for >95% of the total Hg in high trophic level fish (Bloom 1992), total Hg was used as a proxy for MeHg in this study. Bigeye tuna were not sampled in one region (the eastern north Pacific) because the small number of fish captured there precluded having adequate sample sizes for comparison of Hg or Se concentrations. Sampled bigeye tuna

ranged from 2 to 12 kg, while sampled yellowfin tuna ranged from 1 to 44 kg, the majority of which were 1–5 kg (only six yellowfin tuna weighed >10 kg). We pooled the sexes to increase the sample size for our analyses. The second collection effort gathered samples from the fifth study region (the main Hawaiian Islands region) using longline-captured tuna sampled at the United Fishing Agency auction in Hawaii. Longline fisheries generally capture larger-sized tunas than do purse seines. These samples therefore tended to be larger than those in the other four regions. We sampled 50 individuals of each species, ranging from 12 to 20 kg for bigeye tuna and 6–25 kg for yellowfin tuna. We used analysis of variance (ANOVA) and Tukey's tests to determine whether the tuna body sizes significantly differed between regions. Tissue samples from both sampling events were dorsal, white muscle tissue. Individual tuna mass was estimated from length using mass–length regressions (bigeye: Nakamura and Uchiyama 1966, yellowfin: Wild 1994) or from gilled and gutted weights and whole body mass conversions (yellowfin: Zhu et al. 2008, bigeye: Zhu et al. 2009).

We used capture locations of tuna from purse seines to identify their region, as observers recorded individual catch locations and dates. In Hawaii, we obtained capture locations from either the vessel captains or from National Oceanic and Atmospheric Administration logbook data records. Precise individual tuna capture locations were not available in Hawaii; instead, we knew the area in which each fishing vessel operated during trips that captured each sampled tuna.

Total Hg and Se concentrations ($\mu g \cdot g^{-1}$) were measured using closed-vessel microwave digestion with inductively coupled plasma mass spectrometry analysis using a collision cell (Noel et al. 2005; Reyes et al. 2008; Taylor et al. 2008) at the University of Washington's Environmental Health Lab (Seattle, Wash., USA). We analyzed 0.5 g of dried (oven-dried at 50–60 °C), homogenized tissue. The sample was digested using nitric acid and a high-temperature microwave. Quality control measures included National Institute of Standards and Technology one sample of reference material in duplicate for Hg and Se (SRM 1946: Lake Superior Fish Tissue), with mean Hg recoveries of 96.6% and Se recoveries of 158.7%, three spike recovery samples (Hg mean recovery efficiency: 105.2% ± 4.8%; Se mean recovery efficiency: 109.3% ±13.1%) per round of analysis (six total rounds of analysis), standards with varying concentrations of Hg and Se to create calibration curves, and analysis of 47 duplicates (difference between Hg duplicate subsamples: 0.1 ± 0.3 (mean ± SD); Se samples: 0.1 ± 0.3). The detection limit of this method was 0.1 $\mu g \cdot (g \text{ dry mass})^{-1}$. We converted dry mass to wet mass using sample-specific conversion factors recorded during the drying process. The wet mass was 3.6 times the dry mass, on average.

To statistically evaluate regional differences in Hg concentrations, we used a mixed-effects multiple regression model (fixed and random effects) in the R programming environment. This procedure gauged the differences in tuna Hg and Se concentrations between regions while accounting for covariation due to fish mass. Fish mass and region were fixed effects, while fishing trip (Hawaii region) or purse seine haul (other regions) were random effects. The inclusion of a random effect accounts for the covariance shared by fish caught in the same haul. We weighted each model to allow for independent variance estimates by region. Our null model specified that Hg is determined solely by random effects. We used a likelihood ratio test to determine whether a more complicated model that included body size and (or) region was supported by the tuna Hg data (Zuur 2009). The tuna size range sampled in this study was not adequate to test for an interactive effect between body size and region (i.e., a difference in bioaccumulation rates due to differences in growth, reproduction, or other factors among regions). We tested for goodness of fit using the ratio of deviance to residual degrees of freedom (Burnham and Anderson 2002). Our Se model comparisons also included additive model combinations of body size and region.

Results

The five sample regions were distinct relative to DO and had distinct chlorophyll *a* concentrations among the three equatorial regions and the two northern regions, two key oceanographic variables relevant to Hg methylation (Table 1). The eastern north Pacific region reached the lowest DO (<2 $mg \cdot L^{-1}$) at the shallowest depths and had relatively high chlorophyll *a* concentrations. The Hawaiian region had the highest DO at all depths (or the deepest depth at which DO was <2 $mg \cdot L^{-1}$). The three equatorial regions had DO concentrations between these two extremes, with relatively lower DO in the east and progressively higher concentrations to the west. The three equatorial regions had higher mean chlorophyll *a* values integrated over the top 100 m than the two northern regions (two-tailed Student's *t* test, $P < 0.01$).

Yellowfin tuna Hg concentrations varied among and within regions, demonstrating size and location effects. Hg concentrations ranged from 0.03 to 0.7 $\mu g \cdot g^{-1}$ (wet mass), with the highest mean Hg concentrations occurring in the eastern equatorial Pacific region (0.3 $\mu g \cdot g^{-1}$) (Fig. 2). Mean Hg concentrations decreased westward along the equator to 0.1 $\mu g \cdot g^{-1}$ in the central equatorial Pacific. The mean regional values for Hawaii and the eastern north Pacific (0.2 $\mu g \cdot g^{-1}$ for both regions) were similar to each other and less than those in the eastern equatorial region. The mean body size of fish sampled was smaller in the purse seine regions (3.7 kg) than in the Hawaii longline region (15.1 kg). The coefficient of variation for yellowfin tuna Hg concentrations within regions ranged from 0.3 to 0.7.

Like that for yellowfin tuna, bigeye tuna Hg concentrations exhibited substantial variability among regions. Bigeye tuna Hg concentrations were greater than those observed for yellowfin tuna in all regions except the eastern equatorial Pacific, with concentrations in individual samples ranging from undetectable (one sample that we included in the calculations was 0 $\mu g \cdot g^{-1}$) to 1.0 $\mu g \cdot g^{-1}$ across all regions. The highest mean Hg concentrations in this species occurred around Hawaii (0.4 $\mu g \cdot g^{-1}$), where body sizes of sampled fish were greatest (mean 17 kg versus 4.4 kg in the other regions) (Fig. 3). There was also greater variation in Hg values within the Hawaiian region. The Hg concentrations in the three equatorial regions showed a similar trend to that seen for yellowfin tuna, with higher Hg concentrations in the eastern equatorial and lower Hg concentrations in the central equatorial. However, the contrast in bigeye tuna Hg values between

Fig. 2. Yellowfin tuna total Hg concentrations by region (*a*) and by log-transformed body mass (*b*). The boxes represent the median, upper, and lower quartiles, and the whiskers depict the 5% and 95% range. The five regions sampled are the eastern equatorial Pacific (EEP), the mid-equatorial Pacific (MEP), the central equatorial Pacific (CEP), the main Hawaiian Islands (MHI), and the eastern north Pacific (ENP), as indicated in Fig. 1.

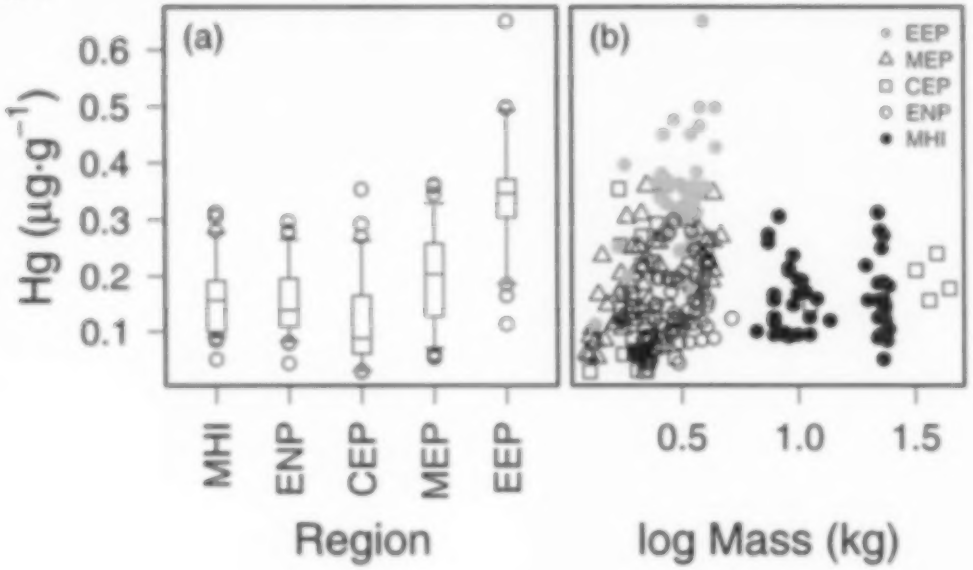
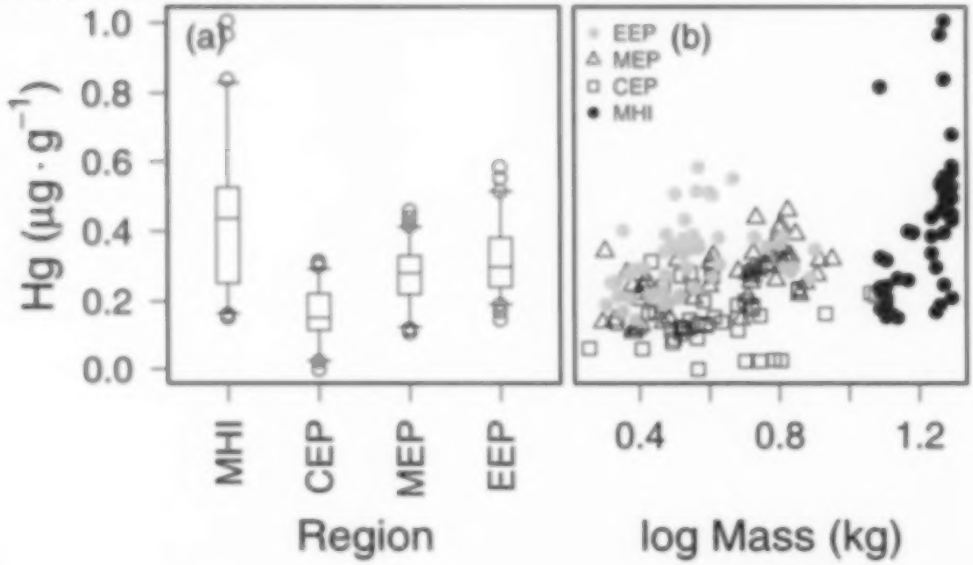


Fig. 3. Bigeye tuna total Hg concentrations by region (*a*) and by log-transformed body mass (*b*). The boxes represent the median, upper, and lower quartiles, and the whiskers depict values that are within the 5% and 95% range (see region definitions in Fig. 2).

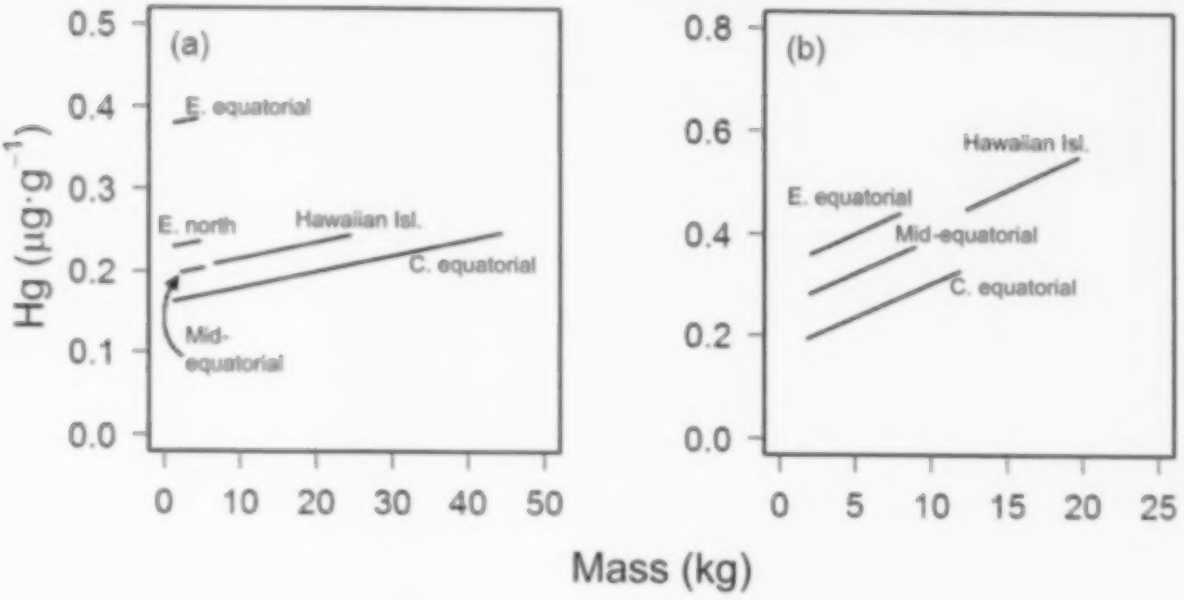


the eastern equatorial and other regions was less than that observed for yellowfin tuna (Fig. 4).

The sizes of tuna available for sampling varied significantly among regions (Table 1; ANOVA yellowfin tuna: $F_{[4,239]} = 44.11$, $p < 0.001$; bigeye tuna: $F_{[3,195]} = 333.9$, $p < 0.001$). These significant differences were mostly due to the larger tuna size sampled from the longline fisheries of the main Hawaiian Islands (Tukey's test of differences between Hawaiian region and all other regions $p < 0.001$ for both species). In addition, the mean size of sampled yellowfin tuna was greater in the central equatorial Pacific than in the mid-equatorial region ($p < 0.05$).

Because of significant variation in body size among regions, our analysis of regional effects on Hg levels must also consider the potential contribution of body size effects. For yellowfin tuna, body size was a significant predictor of Hg

Fig. 4. Predicted Hg concentrations at body mass across each region from the best-fit multiple regression model of Hg in yellowfin tuna (*a*) and bigeye tuna (*b*). Predicted values span the range of sampled body sizes for each region. E. equatorial, eastern equatorial Pacific; Mid-equatorial, mid-equatorial Pacific; C. equatorial, central equatorial Pacific; E. north, eastern north Pacific; Hawaiian Isl., Main Hawaiian Islands.



concentrations in a model without regional effects (Table 2, $p = 0.0422$). Adding region to the model (predicting a unique intercept for each region) significantly improved the model fit (Table 2, $p < 0.0001$), providing support for the hypothesis that differences in Hg concentrations across region were not solely due to differences in body size. In fact, the region with the greatest Hg concentrations (the eastern equatorial Pacific) generally had the smallest body sizes (Fig. 2). Similar results were obtained for bigeye tuna; a model with body mass significantly improved the model over a model with only an intercept tern, and a model with regional effects provided a significant improvement over the model with body mass effects (Table 2). For both species we also reversed the order in which we introduced coefficients into the model, first adding region and then adding body mass. In each test the most complex model (including both mass and region) was the best-fitting model (yellowfin tuna: $p = 0.0223$; bigeye tuna: $p < 0.001$). Notably, because we attempted to sample a standardized size, the range of body sizes within each region was in some cases very small. This data limitation prevented us from fitting more complicated models that tested whether the body mass effect differed across regions. Finally, we reran the models using a more narrow range of tuna body sizes (only tuna with a mass <12 kg), finding the same best-fitting models, strengthening our interpretation that regional differences in Hg concentrations were not due to differences in body size.

For yellowfin tuna, regional-scale effects tended to be larger than the body size effects (Fig. 4). Predicted Hg concentrations varied by more than 50% owing to body size in only two regions (central equatorial and main Hawaiian Islands) (Fig. 4). The fitted model predicts that yellowfin tuna in the eastern equatorial region have higher Hg concentrations than tuna sampled in any other region, regardless of body size, i.e., the small tuna sampled in this region had Hg concentrations that exceeded that predicted from the largest tunas in the other regions. The small-sized tuna sampled in the mid-equatorial Pacific also had elevated Hg levels given their body size. For the other regions, the range of predicted Hg levels expected based on range of sampled body sizes generally overlapped with those differences attributable to regional effects.

Regional-scale differences in Hg concentrations of bigeye tuna were less pronounced, and body size effects were larger than those for yellowfin tuna (Fig. 4). Similar to the yellowfin tuna results, the fitted model predicted that bigeye tuna in the eastern equatorial region have higher Hg concentrations than tuna in any other region. Larger-sized tuna sampled in the main Hawaiian Islands region had higher Hg concentrations than those in the eastern equatorial region, but would have lower Hg concentrations than equivalent-sized tuna from that region.

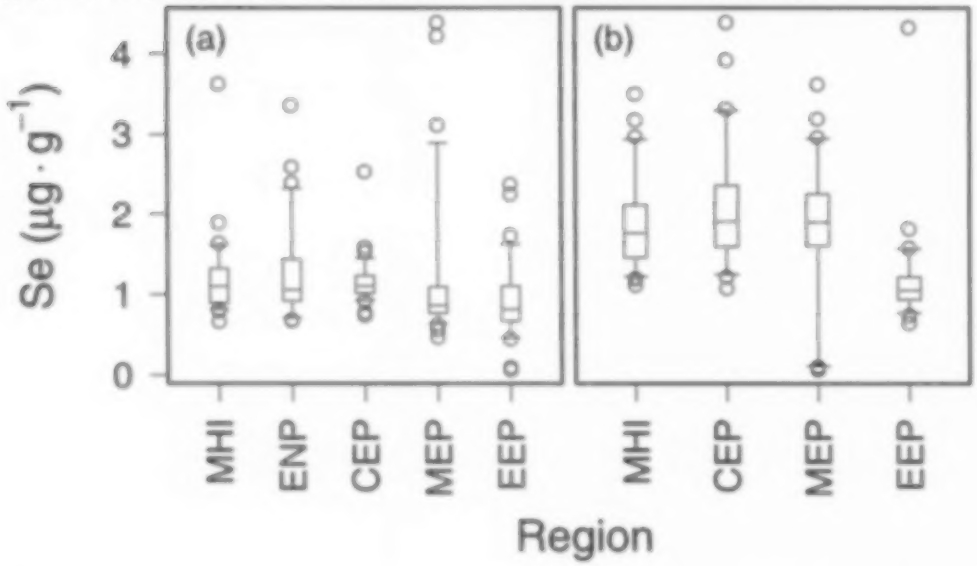
Se concentrations were relatively constant among the regions sampled, ranging from approximately 0 to 3 $\mu g \cdot g^{-1}$ in both species (Fig. 5). Adding a mass factor to the Se null model (intercept only) improved the fit for yellowfin tuna (log-likelihood ratio = 4.3, df = 8, $p = 0.04$) and bigeye tuna (log-likelihood ratio = 4.3, df = 7, $p = 0.04$), but adding a regional factor did not improve the fit for either species (yellowfin tuna: log-likelihood ratio = 1.2, df = 11, $p = 0.7$; bigeye tuna: log-likelihood ratio = 6.8, df = 10, $p = 0.08$), indicating no effect of region on tuna Se concentrations. While Se concentrations for both species always exceeded Hg concentrations (Fig. 5), the Se:Hg ratio increased from east to west across the three equatorial regions.

Table 2. Summary of log-likelihood ratio tests comparing the fit of models to yellowfin and bigeye tuna Hg data.

	Yellowfin		Bigeye	
Model	Log-likelihood ratio	*p* value	Log-likelihood ratio	*p* value
Body mass	4.126	0.0422	21.824	<0.0001
Body mass + region	40.894	<0.0001	15.367	0.0015

Note: Models with body mass and body mass plus region were compared with the null model (intercept only).

Fig. 5. Yellowfin tuna (*a*) and bigeye tuna (*b*) Se concentrations in all sampled regions (see region definitions in Fig. 2). The boxes represent the median, upper, and lower quartiles, and the whiskers depict values that are within the 5% and 95% range.



Discussion

These results support the hypothesis that Hg concentrations in marine apex predators are related to regional oceanographic characteristics that promote the bioavailability of Hg at the base of the food web. We found notable regional-scale variation in tuna Hg concentrations, with the highest concentrations of Hg in both species — after accounting for body size — in the eastern equatorial region, which had relatively high productivity and low DO at the shallowest depths. Tuna Se concentrations were not related to regional oceanographic characteristics, likely reflecting the different bioaccumulation properties of Se (Mason et al. 2000). These patterns suggest that regional differences in biogeochemical cycling of Hg are manifest in apex predators, although further research is required to determine causality. The narrow size range of sampled tunas and the spatially based sampling reduced confounding effects to provide a qualitative verification that bigeye tuna have higher Hg concentrations than yellowfin tuna (Kaneko and Ralston 2007).

The eastern equatorial Pacific was notable as being the single region that was consistently associated with elevated Hg concentrations. If Hg concentrations in apex predators are caused primarily by bottom-up influences operating at the base of the food web, environmental factors affecting Hg cycling (and especially methylation) would be the primary factor causing regional variation in large predatory fishes of similar sizes (e.g., Chen et al. 2000). The current conceptual framework on the bottom-up supply of Hg to marine food webs is based on Mason and Fitzgerald's (1990, 1993) hypothesis that open-ocean methylation rates are dictated by biological productivity and subsequent sinking and remineralization of particulate organic carbon (delivering scavenged Hg to depths with lower oxygen as the carbon is transformed to an inorganic form). Subsequent studies and reviews of the oceanic Hg cycle have supported this view (e.g., Sunderland et al. 2009) while acknowledging the importance and uncertainty of other factors, including availability of inorganic Hg, activity of microorganisms, advective transport of water, demethylation rates, and DO concentrations (Laurier et al. 2004). Our work contributes to this body of knowledge by identifying spatial contrasts in Hg concentrations in apex predators among regions that provide contrasts in the hypothesized processes that regulate Hg methylation. However, in the absence of more extensive (in time and space) sampling, it is difficult to differentiate the relative roles of chlorophyll and DO on Hg concentrations in marine food webs. For instance, the elevated tuna Hg concentrations in the eastern equatorial Pacific corresponded with a combination of

higher primary production and lower DO, whereas other regions with either higher primary production or lower DO (but not both) did not correspond with higher tuna Hg concentrations. The dynamics of the marine Hg cycle could be clarified by measuring Hg concentrations in the water, plankton, and higher trophic levels simultaneously across a gradient of environmental characteristics that affect methylation.

Our analysis provides the first regional, large-scale comparison of fish Hg concentrations in the Pacific. By controlling potentially confounding factors such as size, region, sampling, and analysis techniques, as well as sampling across a gradient of oceanographic conditions hypothesized to govern Hg methylation, our study was unique in being able to identify spatial patterns in tuna Hg concentrations. Other analyses of individual yellowfin and bigeye tuna Hg concentrations, conducted in distinct locations around the Pacific (e.g., Peterson et al. 1973, Choy et al. 2009), produced a larger interregional range of mean Hg values. The lack of controlled factors (e.g., size, region) mentioned above likely contributes to the perceived differences in tuna Hg concentrations found between these studies. Notably, there have been few similar attempts to conduct interregional Hg comparisons, and these have not produced any clear patterns, although they were conducted on smaller regional scales (e.g., Senn et al. 2010). It is possible that our analysis found more striking regional patterns because of the pronounced environmental gradients produced by the eastern boundary currents (Fiedler and Lavin 2006). An even more comprehensive study will be needed to better identify specific oceanographic features responsible for regional-scale patterns in Hg concentrations in tuna.

Although the regional pattern in tuna Hg is consistent with what we would have predicted based on oceanographic conditions, we cannot rule out the possibility that other factors contributed to the observed differences in Hg concentrations. Differences in feeding habits have been found to dictate Hg concentrations in other fish species (McIntyre and Beauchamp 2007), and diet studies confirm that the relative importance of dominant prey items of yellowfin and bigeye tuna varies throughout the Pacific Ocean (e.g., Watanabe 1958; Allain 2004). We do not know whether these diet differences would be expected to produce spatial contrasts in Hg concentrations similar to what we found here. Moreover, fixed spatial features of these regions (e.g., proximity to a source of Hg pollution) (Laurier et al. 2004) and proximity to the continental shelf (Hammerschmidt and Fitzgerald 2006) can also contribute to regional differences. Notably, the Hawaiian region might be expected to have the greatest Hg concentrations owing to an additional source of Hg from ongoing volcanic activity (Davies and Notcutt 1996), but this expectation was not borne out. Yellowfin and bigeye tuna growth rates vary across the Pacific (Shomura 1966), which could also influence their Hg concentrations; however, regional differences in growth rates of these species are not well studied and are often compounded by varying techniques and environmental conditions (Shomura 1966). Higher growth rates could result in decreased Hg concentrations owing to a greater than proportional gain in biomass relative to Hg (Karimi et al. 2007). The tuna sampled in this study are approximately 1–2 years of age, and differences in growth rate are often more apparent in older age classes (e.g., Schaeffer and Fuller 2006).

The regional contrasts in Hg concentrations also support the notion that tunas exhibit a significant degree of regional residency. Tagging studies report estimated dispersal ranges between 700 and 1445 km for yellowfin tuna (Sibert and Hampton 2003; Schaefer et al. 2007) and between 1850 and 1928 km for bigeye tuna (Hampton et al. 1998; Schaefer and Fuller 2005). Geographic variation in growth rates and reproductive biology (Schaefer 1998; Schaeffer et al. 2005; Schaeffer and Fuller 2006) also indicate limited dispersal of individuals and spatial stock structure for these species. The greater contrast in Hg concentrations among regions observed for yellowfin tuna may reflect their smaller dispersal distances and higher regional residence times compared with that of bigeye tuna. If confirmed through more extensive regional sampling, then contaminants may provide a relatively cost effective and rapid way to assess dispersal distances compared with conventional and archival or pop-up satellite tagging methods.

Oceanographic data collected during the time period when the sampled tuna were bioaccumulating Hg (1–2 years) would most directly reflect the conditions influencing the Hg bioavailability experienced by those individuals in this study. Here we used long-term average conditions to reflect persistent contrasts among regions. The eastern and central Pacific regions are influenced by fairly persistent oceanographic features, including the North Pacific Equatorial Countercurrent Province (eastern Pacific warm pool) and the Pacific Equatorial Divergence Province (equatorial cold tongue) (Longhurst 1998), suggesting that conditions promoting MeHg availability might also be persistent. However, environmental variability is well documented on seasonal, interannual (El Niño Southern Oscillation, ENSO), and interdecadal time periods (Pacific Decadal Oscillation) (Fiedler and Lavin 2006), potentially changing the regional Hg cycle. The mean chlorophyll *a* and DO values used to describe each region were based upon long-term data sets, making them robust to periodic fluctuations such as ENSO events. Sampling tuna after a warm or cool phase ENSO event would clarify the extent of the influence of short-term changes in ocean conditions on tuna Hg concentrations.

As some studies call for regulatory agencies to change the basis of fish Hg regulations from solely Hg concentrations to Se:Hg ratios (Kaneko and Ralston 2007), it is important to realize that regional intraspecific variation is apparent in the ratio as well as the individual trace metals. The absence of covariation between Hg and Se likely reflects the different properties of these elements. While Se displays increased concentration in upwelling regions, including the eastern equatorial Pacific (Conde and Alaejos 1997), trophic accumulation of Se is substantially less than that of Hg (Dietz et al. 2000). However, we might not have detected trends in Se concentrations, as this trace metal primarily concentrates in the kidneys and livers of fish (Mason et al. 2000) and we only sampled dorsal white muscle tissue, which is more relevant to consumption regulations. Differences in Se concentration in the water column may not be represented in upper trophic levels owing to the lower rate of trophic transfer. Further sampling of the detoxifying organs would strengthen our observed lack of regional trend in tuna Se concentrations.

Because we found support for the hypothesis that environmental conditions may influence Hg concentrations in commercially important tuna, we sought to estimate the portion of total Pacific Ocean tuna catches that are captured in regions with similar environmental characteristics. These tuna may also have Hg concentrations that are elevated in the same way as those in our sampled eastern equatorial region. We used the World Ocean Database (Boyer et al. 2006) to identify Pacific regions similar in oxygen and chlorophyll *a* properties to our eastern equatorial study region and the Atlas of Tuna and Billfish Catches (http://www.fao.org/fishery/statistics/tuna-atlas/) to obtain mean annual catches from 2000 through 2007 in these regions. On average, approximately 33% of yellowfin tuna and 24% of bigeye tuna caught annually in the Pacific (out of a Pacific-wide, mean annual catch of 741 075 and 224 997 t, respectively) were caught from regions that likely have similar Hg-promoting characteristics to those found in our eastern equatorial region. Thus, a substantial portion of total fishing activities take place in regions where Hg concentrations are anticipated to be higher than average. We note that although the tuna sampled in this study are below concentrations that would trigger more stringent consumption advisories, they do not include the larger tunas captured in so-called dolphin sets or the longline fishery (roughly half of yellowfin tuna and one-quarter of bigeye tuna caught in the eastern Pacific) (Aires-da-Silva and Maunder 2009; Maunder 2009). Thus, a significant portion of total Pacific landings could have elevated Hg concentrations compared to the basin-wide mean concentrations.

The results of this study increase our understanding of the variation in Hg and Se concentrations found in bigeye and yellowfin tuna. The range of intraspecific variation identified within and among regions can be informative in the development and revision of tuna consumption advisories. The US Food and Drug Administration currently reports total mean Hg concentrations in yellowfin and bigeye tuna as 0.325 and 0.639 ppm, with ranges from 0.01 ppm to those exceeding 1 ppm (US Food and Drug Administration 2006). The wide range in concentrations reported at the species level could potentially be decreased if values were stratified spatially, improving the interpretation and application of these concentrations to research and management questions. More precise estimates of tuna Hg concentrations, and models of factors affecting the accumulation of Hg in tuna, will protect humans from exposure to high-Hg fish while removing potential enforcement and marketing obstacles from fishing practices in regions where tuna Hg concentrations are relatively low.

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References

Aires-da-Silva, A., and Maunder, M. 2009. Status of bigeye tuna in the eastern Pacific Ocean in 2008 and outlook. Inter-Amer. Trop. Tuna Comm., Stock Assess. Rep. 8.

Allain, V. 2004. Diet of yellowfin tuna in different areas of the western and central Pacific Ocean. *In* 17th Meeting of the Standing Committee on Tuna and Billfish, Majuro, Marshall Islands, 9–18 August 2004. SCTB Working Paper 17. Western and Western and Central Pacific Fisheries Commission, Noumea, New Caledonia. pp. 1–11.

Bloom, N. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas-chromatography with cold vapor atomic fluorescence detection. Can. J. Fish. Aquat. Sci. **46**(7): 1131–1140. doi:10.1139/f89-147.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49**(5): 1010–1017. doi:10.1139/f92-113.

Boyer, T.P., Antonov, J.I., Garcia, H.E., Johnson, D.R., Locarnini, R.A., Mishonov, A.V., Pitcher, M.T., Baranova, O.K., and Smolyar, I.V. 2006. World Ocean Database 2005 [DVDs]. U.S. Government Printing Office, Washington, D.C.

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information–theoretic approach. Springer, New York, NY.

Chen, C.Y., Stemberger, R.S., Klaue, B., Blum, J.D., Pickhardt, P.C., and Folt, C.L. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. Limnol. Oceanogr. **45**(7): 1525–1536. doi:10.4319/lo.2000.45.7.1525.

Choy, C.A., Popp, B.N., Kaneko, J.J., and Drazen, J.C. 2009. The influence of depth on mercury levels in pelagic fishes and their prey. Proc. Natl. Acad. Sci. U.S.A. **106**(33): 13865–13869. PMID: 19666614.

Conde, J.E., and Alaejos, M.S. 1997. Selenium concentrations in natural and environmental waters. Chem. Rev. **97**(6): 1979–2004. doi:10.1021/cr960100g. PMID:11848896.

Davies, F., and Notcutt, G. 1996. Biomonitoring of atmospheric mercury in the vicinity of Kilauea, Hawaii. Water Air Soil Pollut. **86**(1–4): 275–281. doi:10.1007/BF00279162.

Dietz, R., Riget, F., Cleemann, M., Aarkrog, A., Johansen, P., and Hansen, J.C. 2000. Comparison of contaminants from different trophic levels and ecosystems. Sci. Total Environ. **245**(1–3): 221–231. doi:10.1016/S0048-9697(99)00447-7. PMID:10682369.

Essington, T.E., and Houser, J.N. 2003. The effect of whole-lake nutrient enrichment on mercury concentration in age-1 yellow perch. Trans. Am. Fish. Soc. **132**(1): 57–68. doi:10.1577/1548-8659(2003)132<0057:TEOWLN>2.0.CO;2.

Fiedler, P.C., and Lavin, M.F. 2006. Introduction: A review of eastern tropical Pacific oceanography. Prog. Oceanogr. **69**(2–4): 94–100. doi:10.1016/j.pocean.2006.03.006.

Fitzgerald, W.F., Lamborg, C.H., and Hammerschmidt, C.R. 2007. Marine biogeochemical cycling of mercury. Chem. Rev. **107**(2): 641–662. doi:10.1021/cr050353m. PMID:17300143.

Ganther, H.E., and Sunde, M.L. 2007. Factors in fish modifying methylmercury toxicity and metabolism. Biol. Trace Elem. Res. **119**(3): 221–233. doi:10.1007/s12011-007-8006-6. PMID: 17916945.

Ganther, H.E., Goudie, C., Sunde, M.L., Kopecky, M.J., Wagner, P., Oh, S.H., and Hoekstra, W.G. 1972. Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. Science (Washington, D.C.), **175**(4026): 1122–1124. doi:10.1126/science.175.4026.1122. PMID:5062150.

Hammerschmidt, C.R., and Fitzgerald, W.F. 2006. Methylmercury cycling in sediments on the continental shelf of southern New

England. Geochim. Cosmochim. Acta, **70**(4): 918–930. doi:10.1016/j.gca.2005.10.020.

Hampton, J., Bigelow, K., and Labelle, M. 1998. A summary of current information on the biology, fisheries and stock assessment of bigeye tuna (*Thunnus obesus*) in the Pacific Ocean, with recommendations for data requirements and future research. Secretariat of the Pacific Community, Oceanic Fisheries Programme Technical Report 36.

Hinke, J.T., Kaplan, I.C., Aydin, K., Watters, G.M., Olson, R.J., and Kitchell, J.F. 2004. Visualizing the food-web effects of fishing for tunas in the Pacific Ocean. Ecol. Soc. **9**(1): 10.

Kamykowski, D., and Zentara, S.J. 1990. Hypoxia in the world ocean as recorded in the historical data set. Deep-Sea Res. Part A Oceanogr. Res. Pap. **37**(12): 1861–1874. doi:10.1016/0198-0149(90)90082-7.

Kaneko, J.J., and Ralston, N.V.C. 2007. Selenium and mercury in pelagic fish in the central north pacific near Hawaii. Biol. Trace Elem. Res. **119**(3): 242–254. doi:10.1007/s12011-007-8004-8. PMID:17916947.

Karimi, R., Chen, C.Y., Pickhardt, P.C., Fisher, N.S., and Folt, C.L. 2007. Stoichiometric controls of mercury dilution by growth. Proc. Natl. Acad. Sci. U.S.A. **104**(18): 7477–7482. doi:10.1073/pnas.0611261104. PMID:17456601.

Laurier, F.J.G., Mason, R.P., Gill, G.A., and Whalin, L. 2004. Mercury distributions in the North Pacific Ocean — 20 years of observations. Mar. Chem. **90**(1–4): 3–19. doi:10.1016/j.marchem.2004.02.025.

Levinton, J.S., and Pochron, S.T. 2008. Temporal and geographic trends in mercury concentrations in muscle tissue in five species of Hudson River, USA. Environ. Toxicol. Chem. **27**(8): 1691–1697. doi:10.1897/07-438.1. PMID:18266478.

Longhurst, A.R. 1998. Ecological geography of the sea. Academic Press, San Diego, Calif.

Mann, K.H., and Lazier, J.R.N. 1991. Dynamics of marine ecosystems: biological–physical interactions in the oceans. Blackwell Scientific Publications, Boston.

Mason, R.P., and Fitzgerald, W.F. 1990. Alkylmercury species in the equatorial Pacific. Nature (Lond.), **347**(6292): 457–459. doi:10.1038/347457a0.

Mason, R.P., and Fitzgerald, W.F. 1993. The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean. Deep-Sea Res. Part I Oceanogr. Res. Pap. **40**(9): 1897–1924. doi:10.1016/0967-0637(93)90037-4.

Mason, R.P., Laporte, J.M., and Andres, S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Arch. Environ. Contam. Toxicol. **38**(3): 283–297. doi:10.1007/s002449910038. PMID:10667925.

Maunder, M.N. 2009. Status of yellowfin tuna in the eastern Pacific Ocean in 2008 and outlook. Int.-Am. Trop. Tuna Comm. Stock Assess. Rep. **8**: 3–108.

McIntyre, J., and Beauchamp, D.A. 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Sci. Total Environ. **372**(2–3): 571–584. doi:10.1016/j.scitotenv.2006.10.035. PMID:17157357.

Morel, F.M.M., Kraepiel, A.M.L., and Amyot, M. 1998. The chemical cycle and bioaccumulation of mercury. Annu. Rev. Ecol. Syst. **29**(1): 543–566. doi:10.1146/annurev.ecolsys.29.1.543.

Nakamura, E.L., and Uchiyama, J.H. 1966. Length-weight relations of Pacific tunas. *In* Proceedings of the Governor's Conference on Central Pacific Fishery Resources, Honolulu, Hawaii, 28 February – 12 March 1966. Governor's Conference on Central Pacific Fishery Resources, Honolulu, Hawaii. pp. 197–201.

Nesheim, M.C., and Yaktine, A.L. 2006. Seafood choices: balancing benefits and risks. The National Academies Press, Washington, D.C.

Noel, L., Dufailly, V., Lemahieu, N., Vastel, C., and Guerin, T. 2005. Simultaneous analysis of cadmium, lead, mercury, and arsenic content in foodstuffs of animal origin by inductively coupled plasma/mass spectrometry after closed vessel microwave digestion: Method validation. J. AOAC Int. **88**(6): 1811–1821. PMID:16526466.

Olson, R.J., and Boggs, C.H. 1986. Apex predation by yellowfin tuna (*Thunnus albacares*) — independent estimates from gastric evacuation and stomach contents, bioenergetics, and cesium concentrations. Can. J. Fish. Aquat. Sci. **43**(9): 1760–1775. doi:10.1139/f86-220.

Palacios, D.M., Bograd, S.J., Foley, D.G., and Schwing, F.B. 2006. Oceanographic characteristics of biological hot spots in the North Pacific: a remote sensing perspective. Deep Sea Res. Part II Top. Stud. Oceanogr. **53**(3–4): 250–269. doi:10.1016/j.dsr2.2006.03.004.

Peterson, C.L., Klawe, W.L., and Sharp, G.D. 1973. Mercury in tunas: A review. Fish Bull. **71**(3): 603–613.

Reyes, L.H., Rahman, G.M.M., Fahrenholz, T., and Kingston, H.M.S. 2008. Comparison of methods with respect to efficiencies, recoveries, and quantitation of mercury species interconversions in food demonstrated using tuna fish. Anal. Bioanal. Chem. **390**(8): 2123–2132. doi:10.1007/s00216-008-1966-3. PMID:18317738.

Schaefer, K.M. 1998. Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. Bull. I-ATCC, **21**(6): 205–272.

Schaefer, K.M., and Fuller, D.W. 2005. Conventional and archival tagging of bigeye tuna (*Thunnus obesus*) in the eastern equatorial Pacific Ocean. Col. Vol. Sci. Pap. ICCAT, **57**(2): 67–84.

Schaefer, K.M., Fuller, D.W., and Block, B.A. 2007. Movements, behavior, and habitat utilization of yellowfin tuna (*Thunnus albacares*) in the northeastern Pacific Ocean, ascertained through archival tag data. Mar. Biol. (Berl.), **152**(3): 503–525. doi:10.1007/s00227-007-0689-x.

Schaeffer, K., and Fuller, D. 2006. Estimates of age and growth of bigeye tuna (*Thunnus obesus*) in the Eastern Pacific Ocean based on otolith increments and tagging data. Inter-Amer. Trop. Tuna Comm. **23**(2): 47.

Schaeffer, K., Fuller, D.W., and Miyabe, N. 2005. Reproductive biology of bigeye tuna (*Thunnus obesus*) in the eastern and central Pacific ocean. Inter-Amer. Trop. Tuna Comm. **23**(1): 3–31.

Seki, M.P., Polovina, J.J., Kobayashi, D.R., Bidigare, R.R., and Mitchum, G.T. 2002. An oceanographic characterization of swordfish (*Xiphias gladius*) longline fishing grounds in the springtime subtropical North Pacific. Fish. Oceanogr. **11**(5): 251–266. doi:10.1046/j.1365-2419.2002.00207.x.

Senn, D.B., Chesney, E.J., Blum, J.D., Bank, M.S., Maage, A., and Shine, J.P. 2010. Stable isotope (N, C, Hg) study of methylmercury sources and trophic transfer in the northern Gulf of Mexico. Environ. Sci. Technol. **44**(5): 1630–1637. doi:10.1021/es902361j. PMID:20104887.

Shomura, R.S. 1966. Age and growth studies of four species of tunas in the Pacific Ocean. *In* Proceedings of the Governor's Conference on Central Pacific Fishery Resources, Honolulu, Hawaii, 28 February – 12 March 1966. *Edited by* T.A. Manara. Governor's Conference on Central Pacific Fishery Resources, Honolulu, Hawaii. pp. 203–219.

Sibert, J., and Hampton, J. 2003. Mobility of tropical tunas and the implications for fisheries management. Mar. Policy, **27**(1): 87–95. doi:10.1016/S0308-597X(02)00057-X.

Sunderland, E.M., Krabbenhoft, D.P., Moreau, J.W., Strode, S.A., and Landing, W.M. 2009. Mercury sources, distribution, and bioavailability in the North Pacific Ocean: insights from data and models. Global Biogeochem. Cycles, **23**(2): 14. doi:10.1029/2008GB003425.

Taylor, V.F., Jackson, B.P., and Chen, C.Y. 2008. Mercury speciation and total trace element determination of low-biomass biological samples. Anal. Bioanal. Chem. **392**(7–8): 1283–1290. doi:10.1007/s00216-008-2403-3. PMID:18828006.

Trudel, M., and Rasmussen, J.B. 1997. Modeling the elimination of mercury by fish. Environ. Sci. Technol. **31**(6): 1716–1722. doi:10.1021/es960609t.

US Food and Drug Administration. 2006. Mercury concentrations in fish: FDA Monitoring Program 1990–2010 [online]. Available from http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FoodborneContaminants/MethylMercury/ucm191007.htm [accessed 8 November 2011].

Watanabe, H. 1958. On the difference of the stomach contents of the yellowfin and bigeye tunas from the western equatorial Pacific. Rep. Nankai Reg. Fish. Res. Lab. **7**: 72–81.

Watanabe, H. 1960. Regional differences in food composition of the tunas and marlins from several oceanic areas. Rep. Nankai Reg. Fish. Res. Lab. **12**: 75–83.

Wild, A. 1994. A review of the biology and fisheries for yellowfin tuna, *Thunnus Albacares*, in the Eastern Pacific Ocean. FAO Fish. Tech. Pap. **336**(2): 52–107.

Zhu, G.P., Xu, L.X., Zhou, Y.Q., and Dai, X.J. 2008. Length–frequency compositions and weight–length relations for bigeye tuna, yellowfin tuna, and albacore (Perciformes: Scombrinae) in the Atlantic, Indian, and Eastern Pacific Oceans. Acta Ichthyol. Piscatoria, **38**(2): 157–161. doi:10.3750/AIP2008.38.2.12.

Zhu, G.P., Zhou, Y.Q., Xu, L.X., and Dai, X.J. 2009. Growth and mortality of bigeye tuna *Thunnus obesus* (Scombridae) in the eastern and central tropical Pacific Ocean. Environ. Biol. Fishes, **85**(2): 127–137. doi:10.1007/s10641-009-9471-x.

Zuur, A.F. 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

Response of trout populations in five Colorado streams two decades after habitat manipulation

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Abstract: Evaluating the effectiveness of instream structures for increasing trout populations is complicated by a paucity of long-term studies. We report on a study spanning 23 years to assess the effect of installing log weirs on stream habitat and trout abundance. Structures were installed in a randomly selected half of a 500 m study reach in six small Colorado, USA, mountain streams in 1988, and habitat and trout abundance and biomass were measured annually from 1987 to 1994. When five of the streams were resampled in 2009, none of the 53 logs had moved, and all but one were functioning properly. Pool volume remained more than three times higher in treatment sections than in adjacent controls, and mean depth was also greater. Adult trout abundance increased rapidly after structures were installed and remained 53% higher in treatment sections than in controls 21 years later. Effects on juvenile trout abundance were not detected, probably because fry recruitment is strongly influenced by effects of snowmelt runoff, which vary annually among basins. This evaluation shows that instream structures placed in small, stable channels can function for more than two decades when properly installed and can cause long-lasting increases in trout abundance when habitat is limiting.

Résumé : L'évaluation de l'efficacité des structures ajoutées dans un cours d'eau pour augmenter les populations de poissons est compliquée par l'absence d'études à long terme. Nous présentons une recherche sur 23 années qui mesure l'effet de l'addition de digues en billes de bois sur l'habitat du cours d'eau et l'abondance des truites. Les structures ont été installées dans la moitié (choisie au hasard) de sections d'étude de 500 m dans six petits cours d'eau de montagne du Colorado en 1988 et des mesures de l'habitat, de même que de l'abondance et de la biomasse des truites, ont été faites chaque année de 1987 à 1994. Quand cinq des cours d'eau ont été ré-échantillonnés en 2009, aucune des 53 billes de bois ne s'était déplacée et toutes, sauf une, fonctionnaient adéquatement. Le volume des fosses était encore trois fois plus important dans les sections modifiées que dans les sections témoins adjacentes et la profondeur moyenne était aussi plus grande. L'abondance des truites adultes a augmenté rapidement après l'installation des structures et elle était encore 53 % plus élevée que dans les sections témoins 21 ans plus tard. Aucun effet n'a pu être décelé chez les jeunes truites, probablement parce que le recrutement des alevins est fortement influencé par les effets du ruissellement de la fonte des neiges qui varie chaque année d'un bassin à un autre. Notre évaluation montre que les structures placées dans de petits chenaux stables peuvent fonctionner pendant plus de deux décennies lorsqu'elles sont bien installées et qu'elles peuvent produire des augmentations persistantes de l'abondance des truites lorsque l'habitat constitue un facteur limitant

[Traduit par la Rédaction]

Introduction

Management of stream salmonids often involves manipulation of habitat, under the assumption that increasing physical habitat will increase fish populations (Hunt 1994; Fausch et al. 1995; Whiteway et al. 2010). Addition of instream structures to salmonid streams has been ongoing since the 1930s in North America (White 1996). Numerous studies in various geographic regions have shown that habitat manipulation can increase salmonid abundance and biomass by increasing the quantity and size of pools, overhead cover, and spawning habitat (e.g., Hunt 1976; Gowan and Fausch 1996*a*; Solazzi et al. 2000). However, of four recent reviews, including two with quantitative meta-analyses, two concluded that the addition of instream structures resulted in an increase in salmonid populations (Roni et al. 2008; Whiteway et al. 2010), whereas the other two concluded that evidence for a positive treatment effect was equivocal or lacking (Thompson 2006; Stewart et al. 2009).

The reason some studies failed to detect effects of instream

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structures may be attributed to inadequate experimental design and monitoring. Many prior studies were not true experiments, often lacking adequate controls or replicates, and few used before–after control–impact (BACI) designs that allow robust inference (Stewart-Oaten et al. 1986; Underwood 1994; Manly 2001). In addition, post-manipulation monitoring was frequently too short, often less than 3 years (Whiteway et al. 2010), which is usually insufficient to encompass either long-term changes in stream channel morphology (Kondolf and Micheli 1995) or changes in fish populations in response to habitat changes (Hunt 1976). Thus, recent reviews have pointed out a strong need for long-term monitoring of the stability and effectiveness of instream structures, in part because of reports of high failure rates in certain regions (Frissell and Nawa 1992; House 1996; Roper et al. 1998). Moreover, managers need to know how long any observed increase in fish abundance is sustained, to determine whether habitat manipulation is cost-effective. Finally, most studies did not account for changes in angling that could affect population responses (but see Gowan and Fausch 1996*a*).

Here, we report on habitat and trout populations measured 21 years after installing instream structures, expanding the temporal scope of one of relatively few (and now perhaps the longest) replicated field experiments reported to date. Gowan and Fausch (1996*a*) used a BACI design to test the efficacy of log weirs (also termed log drop structures; see Riley and Fausch 1995 for diagram) at increasing habitat and trout populations in six replicate Colorado mountain streams, and measured these response variables 2 years before and 6 years after treatment. Log weirs are a common habitat manipulation that mimic the effect of large woody debris (LWD) that enters small mountain streams (Richmond and Fausch 1995). By scouring a plunge pool downstream and creating a dammed pool upstream, log weirs increase the volume of pool habitat within a reach (Kozel and Hubert 1989; Fausch and Northcote 1992; Hunt 1994).

Our goal in resampling these streams was to address three specific questions posed by recent reviews and individual studies. First, were the structures long-lasting? Second, did they still create similar habitat conditions more than two decades after installation? Third, was the increase in trout biomass and abundance measured in the first 6 years post-treatment persistent 21 years later?

Background on the original study

Here we present a synopsis of the original experiment that serves as baseline data for our 2009 sampling. Six Colorado mountain streams (2425–2925 m elevation), each with 500 m of relatively homogeneous habitat dominated by riffles and runs, were chosen for study beginning in 1987 (Riley and Fausch 1995; Gowan and Fausch 1996*a*). All are small streams (2.9–3.7 m mean bankfull width before treatment) of moderate gradient (1.2%–2.7%), and four are constrained by relatively narrow bedrock valleys (Jack, Walton, and South St. Vrain creeks and the North Fork Cache la Poudre River). All supported wild populations of primarily brook trout (*Salvelinus fontinalis*) or brown trout (*Salmo trutta*), or a mixture of brook, brown, and rainbow trout (*Oncorhynchus mykiss*). To supply wood for railroad ties and mining timbers, channels of many streams in this region were cleared of LWD and boulders 75–100 years before to allow for the transportation of logs cut from riparian zones and uplands (Young et al. 1994; Fausch and Young 2004). This reduction in LWD and boulders resulted in channels with relatively few pools. Total volume of LWD measured in 1993 in eight streams of similar size traversing old-growth forest was 4.2 times higher than that measured in the control sections of four of the six study streams (see below; all except Little Beaver and South St. Vrain creeks). In addition, density of LWD pieces was 2.2 times higher, indicating that natural wood loads are much higher in reaches unaffected by timber harvest (Richmond and Fausch 1995).

In each study stream, 10 log weirs were constructed in late summer 1988 in a randomly chosen 250 m half of the study reach, and these structures were maintained until 1994. One stream (Little Beaver Creek) had 60 m of divided channel in the treatment reach, so double the number of shorter logs were installed in this section (six instead of three, totaling 13 logs) to create a similar habitat volume as in other streams. Habitat and fish abundance and biomass were measured in each stream for multiple years before (1987–1988) and after (1989–1994) logs were installed. Mechanisms responsible for changes in fish abundance and biomass (i.e., growth, survival, recruitment, movement, and angling pressure) were also evaluated through focused studies (Riley et al. 1992; Gowan and Fausch 1996*a*; Latterell et al. 1998).

Abundance of juvenile (age 1) and adult trout (age 2 and older) in treatment and control sections were approximately equal and not significantly different before logs were installed. After installation, treatment sections contained an average of 42% more adult trout than control sections, a significant increase, and results were similar for biomass (Gowan and Fausch 1996*a*; see online Supplementary Table S1[1] for data from the original study). In contrast, abundance and biomass of juveniles were not significantly affected by the structures. Following log installation, pool habitat increased rapidly and remained stable, increasing by an average of 520% in treatment sections during the final 4 years (1991–1994). Mean depth was also higher in treatment than in control sections post-treatment (not measured pre-treatment), but wetted area did not increase.

The increase in trout abundance and biomass in all streams occurred rapidly, generally within 1 year, and was primarily driven by immigration into treatment reaches rather than changes in growth, recruitment, survival, or angling pressure (Riley et al. 1992; Gowan and Fausch 1996*a*, 1996*b*). This fish movement took place over relatively large spatial scales (hundreds to thousands of metres), as few immigrants into treatment sections came from adjacent control sections. Treatment effects persisted throughout the 6 years of post-installation monitoring, by which time immigration and emigration rates in the treatment and control reaches had approximately equalized, but with a higher "equilibrium" trout abundance in the treatment reaches.

Materials and methods

In July 2009, we resampled five of the six streams (Colo-

[1]Supplementary data are available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/f2011-125).

rado, Jack, Little Beaver, and Walton creeks and the North Fork Cache la Poudre River) to determine whether effects of log weirs on habitat and trout populations persisted 21 years after installation. We were unable to resample South St. Vrain Creek owing to logistical constraints. To assess stability of log structures, we classified them into one of three movement categories after Roper et al. (1998): (1) in place, (2) shifted on site, or (3) removed from the site. For structures in the first two categories, we determined whether they continued to form pools, or whether water flowed underneath or around logs and hence failed to form either a dammed pool above or a plunge pool below. Annual peak flows are usually created by snowmelt runoff in this region (Hauer et al. 1997), so we analyzed the recurrence intervals of annual floods (Dunne and Leopold 1978) at the nearest gauge during the post-treatment period (1989–1994) and afterwards (1995–2009) to assess the relative magnitude of disturbances influencing structure stability.

Abundance and biomass of age-1 and age-2 and older trout, pool volume, mean depth, and wetted surface area were measured using the same methods as those of Gowan and Fausch (1996*a*). Four streams contained brook trout (Jack, Walton, and Colorado creeks and the North Fork Cache la Poudre River), and the fifth (Little Beaver Creek) supported brown trout. A small proportion of cutthroat trout was captured in Jack Creek, and brook trout in Little Beaver Creek, but these were excluded as in the previous analysis. Lengths of the two age groups of trout overlapped little in each stream, so we separated them using stream-specific length–frequency histograms. Kennedy et al. (2003) showed that most female brook trout matured at age 2 (based on otoliths) in a northern Colorado stream at similar elevation (2680 m), so our age classes generally separated juveniles versus adults. Maximum life span in that stream was age 5, but reached age 14 in another stream at higher elevation (3200 m), so adults in our streams likely comprised at least 3 year classes (ages 2–4).

Fish abundance and biomass for each age class were estimated using a maximum-likelihood removal estimator in CAPTURE (White et al. 1982) based on three-pass backpack electrofishing. Each treatment and control section was blocked at the ends with seines, and all passes within a section were completed in 1 day. Gowan and Fausch (1996*a*) found this method to be accurate based on a validation using marked fish. We also analyzed these data with the newer methods in MARK described in White and Burnham (1999) and White (2008), but owing to high capture probabilities for age-1 and older fish, the results were essentially identical ($r \geq 0.99$, $P < 0.0001$ for both age classes), so we used the original estimator to allow direct comparison to earlier analyses. Habitat parameters were estimated using transects spaced every 5 m (depth and area) or 1 m (pool volume), as before.

For each response variable, we fit a mixed model analysis of variance (ANOVA) using restricted maximum likelihood (REML) with a random effect for stream and fixed effects for stream section (treatment or control), time period (pre-treatment 1987–1988, post-treatment 1989–1994, and post-treatment in 2009), and the section × period interaction. Data for each stream section were first averaged within each of the first two periods, such that each contributed one data point per time period. This provided five replicates (streams) for each period and made accounting for temporal autocorrelation within periods unnecessary (see Gowan and Fausch 1996*a* for a detailed analysis of temporal autocorrelation within the first two periods). For the habitat variables, pre-treatment data were only from 1987 because measurements were not made in treatment sections in 1988 before logs were installed. In addition, post-treatment habitat data were averaged over the final 4 of 6 years (1991–1994), to assess habitat changes at their maximum extent during this period.

To account for additional autocorrelation and unequal variance among periods within a stream section, we considered three covariance structures (Littell et al. 2006): (1) variance components (the default), (2) unstructured, and (3) autoregressive (lagged one period). For each response variable, we used the covariance structure with the lowest AIC_c value (Akaike's information criterion, corrected for small samples), which selected the model best supported by the data. The autoregressive model was never supported. The degrees of freedom to test main effects and contrasts were higher for the variance components than for the unstructured model (e.g., df = 20 vs. 8 for contrasts) because of the variances and covariances estimated.

If a treatment effect was present, there would be a significant interaction between section and period (e.g., an increase in trout abundance in treatment sections but not controls), so we used a priori contrasts to test two specific hypotheses. For each response variable, we first tested whether the difference between the treatment and control sections was greater in 2009 than during the pre-treatment (1987–1988) period. We then used the second contrast to test whether the magnitude of the treatment effect measured in 2009 was different than the magnitude measured during the initial post-treatment period (1989–1994). We also used a third contrast to test whether mean trout abundance and biomass had changed from the post-treatment period to 2009, averaging across both treatment and control sections.

Before analysis, we log-transformed juvenile and adult trout biomass to correct for heterogeneous variance. We adjusted the denominator degrees of freedom for all tests using the Kenward–Rogers method, which accounts for estimation of additional variance and covariance parameters and makes these tests relatively conservative (Littell et al. 2006). That is, treatment effects would have to be relatively large to be declared significant. Analyses of variance were fit using PROC Mixed in SAS 9.2 (SAS Institute Inc. 2008).

Results

Longevity of instream structures

All 53 logs installed in the five study streams in 1988 were located in 2009 and were in place, and all but one (98%) were functioning to create dammed and plunge pools. For one log, bank erosion on one end resulted in flow around rather than over the log, but it had not moved and still formed a small pool downstream. Three other log structures showed signs of imminent failure, one from bank erosion and two from rusting hardware cloth on the upstream side. Therefore, 49 of the 53 logs were in the same condition as in 1994, despite no maintenance during the intervening 15 years. Overall, the log structures had been exposed to relatively low annual peak flows during the 6 years after con-

struction (1989–1994), all of which were 1.0- to 4.5-year recurrence interval floods at the nearest gauges. However, in the years afterwards (1995–2009) structures were exposed to the largest floods on record in two river basins (35-year recurrence for Walton Creek; 36-year recurrence for Little Beaver Creek and North Fork Cache la Poudre River; see Riley and Fausch 1995 for map), and a flood in the top 10th percentile in the third (12-year recurrence; Jack and Colorado creeks).

Effects of structures on habitat

Pool volume averaged 229% higher in treatment than in control sections during 2009, a significant increase over pre-treatment conditions (by a priori contrast after ANOVA, $t_{20} = 4.40$, $P = 0.0003$; Fig. 1) but not significantly different than the increase measured during the post-treatment period from 1991 to 1994 ($t_{20} = -0.60$, $P = 0.55$). Mean depth was also greater in treatment than in control sections during 2009, similar to the post-treatment period (difference of 0.04 ± 0.017 m (mean ± standard error (SE)) in 2009 vs. 0.06 ± 0.009 m post-treatment; $F_{[1,8]} = 0.31$, $P = 0.60$ for the section × period interaction for these two periods). Wetted area, which did not increase after logs were installed (Gowan and Fausch 1996*a*), was not significantly different in 2009 compared with that in either the pre-treatment ($t_{20} = 0.20$, $P = 0.84$) or post-treatment ($t_{20} = -0.60$, $P = 0.56$) periods.

Effects of structures on fish abundance and biomass

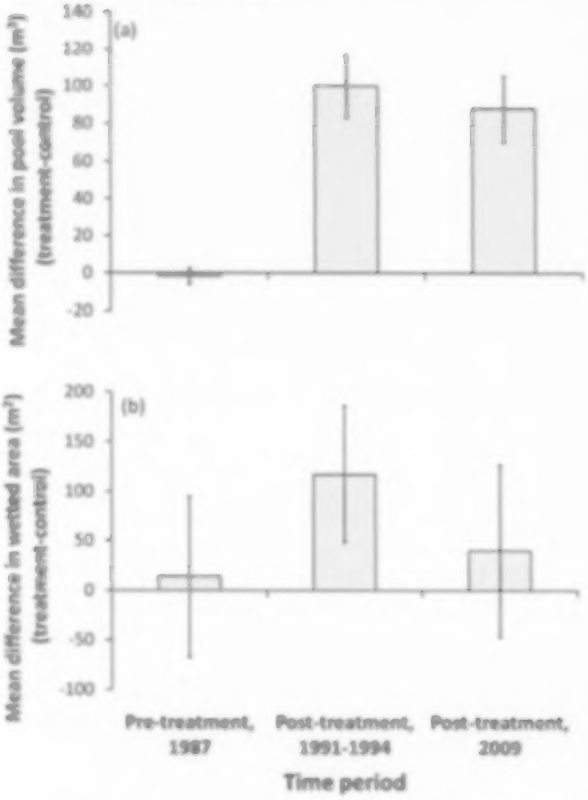
Treatment sections had an average of 53% more adult trout than control sections in 2009, and this was a significant increase compared with the pre-treatment period for both abundance and biomass (by a priori contrast after ANOVA: $t_8 = 2.36$, $P = 0.046$ for abundance; $t_{20} = 2.23$, $P = 0.04$ for biomass; Fig. 2). The effect of the treatment on adult trout was not significantly different in 2009 from that in the post-treatment period from 1989 to 1994 (based on the contrast of the two periods: $t_8 = -0.95$, $P = 0.37$ for abundance; $t_{20} = 0.35$, $P = 0.73$ for biomass), even though adult trout abundance averaged over treatment and control sections was 50% (SE = 6.1%) lower in 2009 than during the post-treatment period ($t_8 = 5.43$, $P = 0.0006$ for abundance; $t_{20} = 4.32$, $P = 0.0003$ for biomass).

In contrast, installing the structures did not significantly increase juvenile abundance or biomass in 2009 compared with that during the pre-treatment ($t_{20} = 0.08$, $P = 0.93$ for abundance; $t_8 = 0.37$, $P = 0.72$ for biomass) or immediate post-treatment periods ($t_{20} = -0.77$, $P = 0.45$ for abundance; $t_8 = -0.34$, $P = 0.74$ for biomass). Likewise, mean juvenile abundance changed little in 2009 compared with that during the post-treatment period, but was highly variable (1.0% change, SE = 25.5%; $t_{20} = 1.29$, $P = 0.21$ for abundance; $t_8 = -0.86$, $P = 0.42$ for biomass).

Discussion

The instream structures we tested proved to be durable and stable for more than two decades and continued to support higher habitat volume and adult trout abundance and biomass in treatment reaches. In contrast, we could detect no effect of installing the log structures on juvenile trout abundance and biomass. Analysis of the original data (Latterell et al. 1998), and research by others (Nehring and Anderson 1993),

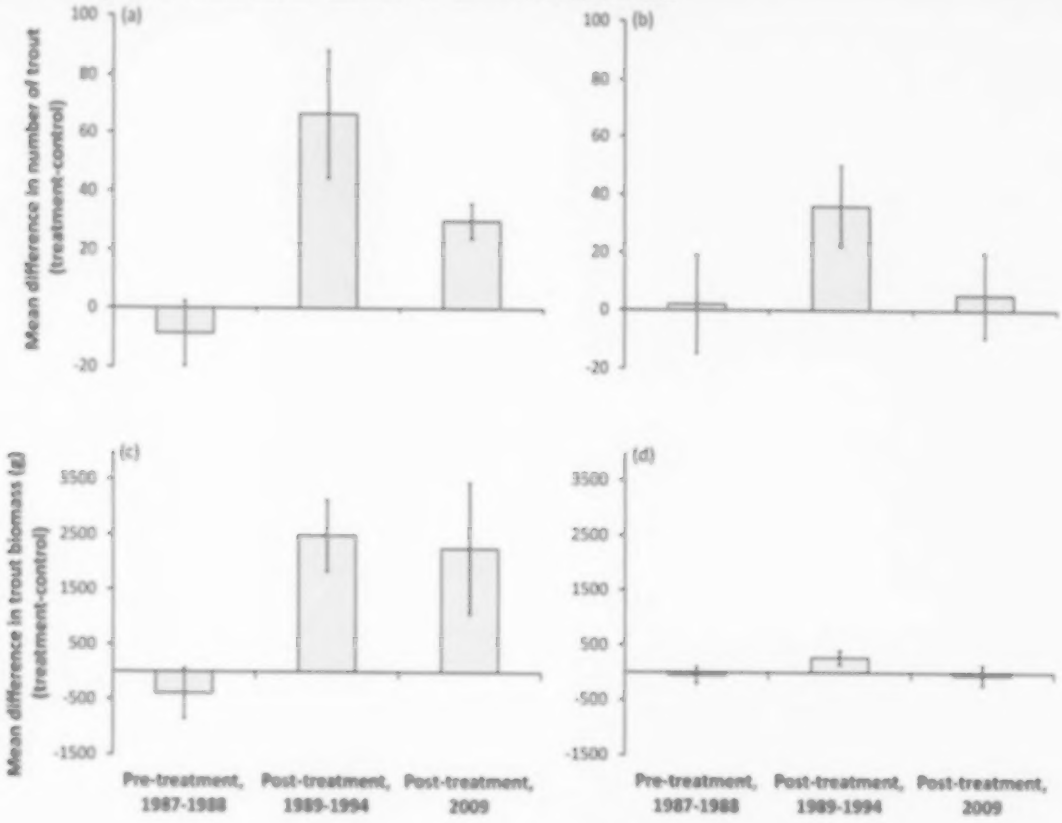
Fig. 1. Mean difference between treatment and control sections for (*a*) pool volume and (*b*) wetted area in five study streams across three study periods (pre-treatment, post-treatment during 1991–1994, and post-treatment during 2009). Vertical bars indicate ±1 SE, calculated from the raw data.



showed that juvenile trout recruitment in these mountain streams is significantly reduced by high snowmelt runoff flows the previous year when fry emerge, and these flows can vary among river basins. Mean June flows (the month of highest snowmelt runoff) in 2008 were the second highest on record in one of the three river basins in which the study streams were located, but at the 75th and 50th percentile in the other two river basins. Therefore, it is plausible that this regional variation in snowmelt runoff had strong effects on this single age class, reducing recruitment in different streams in different years and preventing detection of consistent effects of instream structures on age-1 trout across streams.

Studies on the long-term efficacy of instream structures often question their longevity, especially if not maintained regularly (Frissell and Nawa 1992; White 1996). In an early study in a California mountain stream, an environment similar to that of the streams we studied, Ehlers (1956) evaluated 41 log weirs and deflectors 18 years after installation and found multiple failures owing to improper installation, bank erosion, and substrate movement. However, in studies conducted in coastal Pacific Northwest streams subject to winter floods capable of restructuring channels, >80% of structures remained in place and functioning, at least within about 10 years after placement (Roper et al. 1998; Roni et al.

Fig. 2. Mean difference between treatment and control sections for (*a*) abundance of adult trout, (*b*) abundance of juveniles, (*c*) biomass of adults, and (*d*) biomass of juveniles in five study streams across three study periods (pre-treatment, post-treatment during 1989–1994, and post-treatment during 2009). Vertical bars indicate ±1 SE, calculated from the raw data.



2002). Binns (1994) found similar results in Rocky Mountain streams. We attribute the longevity of the log structures in the central Rocky Mountain streams we studied to three factors. First, most had plane-bed and step-pool channels of moderate gradient with substantial cobble and boulder substrate, which are highly stable despite annual snowmelt runoff (Montgomery and Buffington 1997). Second, peak runoff flows were relatively low for the 6 years after logs were installed (1989–1994), allowing the bed and banks to stabilize before subsequent years of high peak flows. Third, logs weirs were installed by US Forest Service field crews supervised by personnel experienced in building the structures, and we maintained them from 1989 through 1994.

In addition to remaining in place, the instream structures continued to more than triple pool habitat volume in the treatment sections 21 years after installation. Similarly, Whiteway et al. (2010) reported a mean increase in pool area of 1.9 times (calculated from their effect size), and an increase in depth of 1.3 times, based on a meta-analysis of 211 instream restoration projects (primarily cover structures, deflectors, and weirs). However, we found no evidence for an increase in wetted area in 2009 or previously (Gowan and Fausch 1996*a*), probably because three of five streams are constrained by bedrock valleys and have channel banks that are not overtopped by the pools created by logs.

The conclusion in two recent reviews that habitat manipulation often fails to increase fish populations (Thompson 2006; Stewart et al. 2009, but see Whiteway et al. 2010) raises the question of why a strong and persistent treatment effect occurred in the streams we studied. Two main processes probably account for these effects. First, adult trout habitat (physical space) was a main limiting factor in these streams, and the pool volume created by the structures was sufficient to increase the carrying capacity of the habitat (e.g., Fausch and Northcote 1992). Past logging practices contributed to low LWD densities in these streams (Richmond and Fausch 1995), and habitat consisted of mostly riffles and runs (Young et al. 1994; Fausch and Young 2004) with few pools to support adult trout before logs were installed. Given this, increasing mean pool volume in treatment sections from less than 20 m^3 to more than 125 m^3 was sufficient to produce a large increase in adult trout. In contrast, a response by juvenile trout could not be detected, apparently because they were limited by snowmelt runoff flows that cause high fry mortality and vary by region and among years. However, juveniles of other salmonids have responded

positively to log or boulder structures that increased pool volume in other regions (Roni and Quinn 2001*a*; Roni et al. 2006). A second reason that we found a persistent treatment effect is because channels were stable, allowing pool habitat created by logs to persist for decades. Other researchers also reported that structures installed in streams with large substrate or constrained channels, and anchored to one or both banks, can persist for long periods (Binns 1994; Roper et al. 1998; Roni et al. 2006).

Annual fluctuations in trout abundance can also confound detection of treatment effects. A strong treatment effect persisted in 2009 despite a large reduction in mean adult trout abundance, indicating that habitat manipulation at local scales can be effective despite larger-scale regional influences such as droughts and harsh winters (Gowan and Fausch 1996*a*). Vehanen et al. (2010) found a similar positive treatment effect of log and boulder structures on adult brown trout populations in Finland, but they considered the structures a failure because abundance in both treatments and controls declined during the post-treatment period owing to a drought. Because they reported large differences in adult trout density in treatments compared with that in controls for five of seven samples over two post-treatment years, we believe a more appropriate interpretation is that their habitat manipulations produced a positive response despite drought.

Several studies indicate that the rapid increase in trout abundance following habitat enhancement is driven by immigration (Gowan and Fausch 1996*a*; Roni and Quinn 2001*b*). Other research indicates that the mobile individuals driving these responses are often large dominant fish seeking more complex habitat (Harvey et al. 1999; Kahler et al. 2001) or more profitable feeding positions (Gowan 2007) across various spatial scales. For example, Hansen and Closs (2009) found that the second- and third-ranked galaxiids in pool dominance hierarchies of a New Zealand stream moved most often, and moved farther than smaller subordinates. They speculated that these relatively large subdominant fish needed to travel farther to find suitable habitat given their higher energy requirements. This conclusion is supported by Gowan and Fausch (2002), who reported that dominant fish, when experimentally denied access to their preferred foraging locations, exited pools altogether rather than simply occupying the next-best position. Similarly, at the population level, Gowan and Fausch (1996*b*) found that trout that moved were the larger adults in poorer condition. It is also logical to conclude that smaller subordinates, which move less (Hansen and Closs 2009), will fill locations vacated by larger emigrants, thereby contributing to a stream-wide increase in trout abundance.

To our knowledge, this study is the longest and among the most comprehensive evaluation of instream habitat structures published to date. Five others that evaluated structures after 15–20 years also showed significant positive responses by salmonids (see references in Whiteway et al. 2010). However, we caution that managers must consider stream geomorphology, the effectiveness of specific structures in ameliorating limiting factors of habitat, and the ecology and life history of the fishes involved when planning stream habitat enhancement. We conclude that installing log structures caused substantial and long-term increases in adult trout abundance and biomass in these streams because stream channels were stable, which allowed the structures to continue functioning to create pools, and because these pools were a limiting resource for adult trout.

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References

Binns, N.A. 1994. Long-term responses of trout and macrohabitats to habitat management in a Wyoming headwater system. N. Am. J. Fish. Manage. **14**(1): 87–98. doi:10.1577/1548-8675(1994)014<0087:LTROTA>2.3.CO;2.

Dunne, T., and Leopold, L.B. 1978. Water in environmental planning. W.H. Freeman, New York.

Ehlers, R. 1956. An evaluation of stream improvement devices constructed eighteen years ago. Calif. Fish Game, **42**(3): 203–217.

Fausch, K.D., and Northcote, T.G. 1992. Large woody debris and salmonid habitat in a small coastal British Columbia stream. Can. J. Fish. Aquat. Sci. **49**(4): 682–693. doi:10.1139/f92-077.

Fausch, K.D., and Young, M.K. 2004. Interactions between forests and fish in the Rocky Mountains of the USA. *In* Fishes and forestry: worldwide watershed interactions and management. *Edited by* T.G. Northcote and G.F. Hartman. Blackwell Science, Oxford, UK. pp. 463–484.

Fausch, K.D., Gowan, C., Richmond, A.D., and Riley, S.C. 1995. The role of dispersal in trout population response to habitat formed by large woody debris in Colorado mountain streams. Bull. Fr. Peche Piscic. **337/338**(339): 179–190. doi:10.1051/kmae:1995020.

Frissell, C.A., and Nawa, R.K. 1992. Incidence and causes of physical failure of artificial habitat structures in streams of western Oregon and Washington. N. Am. J. Fish. Manage. **12**(1): 182–197. doi:10.1577/1548-8675(1992)012<0182:IACOPF>2.3.CO;2.

Gowan, C. 2007. Short-term cues used by foraging trout in a California stream. Environ. Biol. Fishes, **78**(4): 317–331. doi:10.1007/s10641-006-9099-z.

Gowan, C., and Fausch, K.D. 1996*a*. Long-term demographic responses of trout populations to habitat manipulation in six Colorado streams. Ecol. Appl. **6**(3): 931–946. doi:10.2307/2269496.

Gowan, C., and Fausch, K.D. 1996*b*. Mobile brook trout in two high-elevation Colorado streams: re-evaluating the concept of restricted movement. Can. J. Fish. Aquat. Sci. **53**(6): 1370–1381. doi:10.1139/f96-058.

Gowan, C., and Fausch, K.D. 2002. Why do foraging stream salmonids move during summer? Environ. Biol. Fishes, **64**(1–3): 139–153. doi:10.1023/A:1016010723609.

Hansen, E.A., and Closs, G.P. 2009. Long-term growth and movement in relation to food supply and social status in a stream fish. Behav. Ecol. **20**(3): 616–623. doi:10.1093/beheco/arp039.

Harvey, B.C., Nakamoto, R.J., and White, J.L. 1999. Influence of large woody debris and a bankfull flood on movement of adult resident coastal cutthroat trout (*Oncorhynchus clarki*) during fall and winter. Can. J. Fish. Aquat. Sci. **56**(11): 2161–2166. doi:10.1139/f99-154.

Hauer, F.R., Baron, J.S., Campbell, D.H., Fausch, K.D., Hostetler, S. W., Leavesley, G.H., Leavitt, P.R., McKnight, D.M., and Stanford, J.A. 1997. Assessment of climate change and freshwater ecosystems of the Rocky Mountains, U.S., and Canada. Hydrol. Process. **11**(8): 903–924. doi:10.1002/(SICI)1099-1085(19970630)11:8<903::AID-HYP511>3.0.CO;2-7.

House, R.A. 1996. An evaluation of stream restoration structures in a coastal Oregon stream 1981–1993. N. Am. J. Fish. Manage. **16**(2): 272–281. doi:10.1577/1548-8675(1996)016<0272:AEOSRS>2.3.CO;2.

Hunt, R.L. 1976. A long-term evaluation of trout habitat development and its relation to improving management-related research. Trans. Am. Fish. Soc. **105**(3): 361–364. doi:10.1577/1548-8659(1976)105<361:ALEOTH>2.0.CO;2.

Hunt, R.L. 1994. Trout stream therapy. The University of Wisconsin Press, Madison, Wis.

Kahler, T.H., Roni, P., and Quinn, T.P. 2001. Summer movement and growth of juvenile anadromous salmonids in small western Washington streams. Can. J. Fish. Aquat. Sci. **58**(10): 1947–1956. doi:10.1139/f01-134.

Kennedy, B.M., Peterson, D.P., and Fausch, K.D. 2003. Different life histories of brook trout populations invading mid-elevation and high-elevation cutthroat trout streams in Colorado. West. N. Am. Nat. **63**(2): 215–223.

Kondolf, G.M., and Micheli, E.R. 1995. Evaluating stream restoration projects. Environ. Manage. **19**(1): 1–15. doi:10.1007/BF02471999.

Kozel, S.J., and Hubert, W.A. 1989. Factors influencing the abundance of brook trout (*Salvelinus fontinalis*) in forested mountain streams. J. Freshwat. Ecol. **5**(1): 113–122. doi:10.1080/02705060.1989.9665219.

Latterell, J.J., Fausch, K.D., Gowan, C., and Riley, S.C. 1998. Relationship of trout recruitment to snowmelt runoff flows and adult trout abundance in six Colorado mountain streams. Rivers, **6**(4): 240–250.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., and Schabenberger, O. 2006. SAS for mixed models. 2nd ed. SAS Institute Inc., Cary, N.C.

Manly, B.F.J. 2001. Statistics for environmental science and management. Chapman and Hall, New York.

Montgomery, D.R., and Buffington, J.M. 1997. Channel reach morphology in mountain drainage basins. Geol. Soc. Am. Bull. **109**(5): 596–611. doi:10.1130/0016-7606(1997)109<0596:CRMIMD>2.3.CO;2.

Nehring, R.B., and Anderson, R.M. 1993. Determination of population-limiting critical salmonid habitats in Colorado streams using the Physical-Habitat Simulation System. Rivers, **4**(1): 1–19.

Richmond, A.D., and Fausch, K.D. 1995. Characteristics and function of large woody debris in subalpine Rocky Mountain streams of northern Colorado. Can. J. Fish. Aquat. Sci. **52**(8): 1789–1802. doi:10.1139/f95-771.

Riley, S.C., and Fausch, K.D. 1995. Trout population response to habitat enhancement in six northern Colorado streams. Can. J. Fish. Aquat. Sci. **52**(1): 34–53. doi:10.1139/f95-004.

Riley, S.C., Fausch, K.D., and Gowan, C. 1992. Movement of brook trout (*Salvelinus fontinalis*) in four small subalpine streams in northern Colorado. Ecol. Freshwat. Fish, **1**(2): 112–122. doi:10.1111/j.1600-0633.1992.tb00080.x.

Roni, P., and Quinn, T.P. 2001*a*. Density and size of juvenile salmonids in response to placement of large woody debris in western Oregon and Washington streams. Can. J. Fish. Aquat. Sci. **58**(2): 282–292. doi:10.1139/f00-246.

Roni, P., and Quinn, T.P. 2001*b*. Effects of wood placement on movements of trout and juvenile coho salmon in natural and artificial stream channels. Trans. Am. Fish. Soc. **130**(4): 675–685. doi:10.1577/1548-8659(2001)130<0675:EOWPOM>2.0.CO;2.

Roni, P., Beechie, T.J., Bilby, R.E., Leonetti, F.E., Pollock, M.M., and Pess, G.R. 2002. A review of stream restoration techniques and a hierarchical strategy for prioritizing restoration in Pacific Northwest watersheds. N. Am. J. Fish. Manage. **22**(1): 1–20. doi:10.1577/1548-8675(2002)022<0001:AROSRT>2.0.CO;2.

Roni, P., Bennett, T., Morely, S., Pess, G.R., Hanson, K., Van Slyke, D., and Olmstead, P. 2006. Rehabilitation of bedrock stream channels: the effects of boulder weir placements on aquatic habitat and biota. River Res. Appl. **22**(9): 967–980. doi:10.1002/rra.954.

Roni, P., Hanson, K., and Beechie, T. 2008. Global review of the physical and biological effectiveness of stream habitat rehabilitation techniques. N. Am. J. Fish. Manage. **28**(3): 856–890. doi:10.1577/M06-169.1.

Roper, B.B., Konnoff, D., Heller, D., and Wieman, K. 1998. Durability of Pacific Northwest instream structures following floods. N. Am. J. Fish. Manage. **18**(3): 686–693. doi:10.1577/1548-8675(1998)018<0686:DOPNIS>2.0.CO;2.

SAS Institute Inc. 2008. Statistical Analysis System, version 9.2 [computer program]. SAS Institute Inc., Cary, N.C.

Solazzi, M.F., Nickelson, T.E., Johnson, S.L., and Rodgers, J.D. 2000. Effects of increasing winter rearing habitat on abundance of salmonids in two coastal Oregon streams. Can. J. Fish. Aquat. Sci. **57**(5): 906–914. doi:10.1139/f00-030.

Stewart, G.B., Bayliss, H.R., Showler, D.A., Sutherland, W.J., and Pullin, A.S. 2009. Effectiveness of engineered in-stream structure mitigation measures to increase salmonid abundance: a systematic review. Ecol. Appl. **19**(4): 931–941. doi:10.1890/07-1311.1. PMID:19544735.

Stewart-Oaten, A., Murdoch, W.W., and Parker, K.R. 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology, **67**(4): 929–940. doi:10.2307/1939815.

Thompson, D.M. 2006. Did the pre-1980 use of in-stream structures improve streams? a reanalysis of historical data. Ecol. Appl. **16**(2): 784–796. doi:10.1890/1051-0761(2006)016[0784:DTPUOI]2.0.CO;2. PMID:16711062.

Underwood, A.J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecol. Appl. **4**(1): 3–15. doi:10.2307/1942110.

Vehanen, T., Huusko, A., Mäki-Petäys, A., Louhi, P., Mykrä, H., and Muotka, T. 2010. Effects of habitat rehabilitation on brown trout (*Salmo trutta*) in boreal forest streams. Freshw. Biol. **55**(10): 2200–2214. doi:10.1111/j.1365-2427.2010.02467.x.

White, R.J. 1996. Growth and development of North American stream habitat management for fish. Can. J. Fish. Aquat. Sci. **53**(Suppl. 1): 342–363. doi:10.1139/f96-044.

White, G.C. 2008. Closed population estimation models and their extensions in Program MARK. Environ. Ecol. Stat. **15**(1): 89–99. doi:10.1007/s10651-007-0030-3.

White, G.C., and Burnham, K.P. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study, **46**(Suppl.): 120–139. doi:10.1080/00063659909477239.

White, G.C., Anderson, D.R., Burnham, K.P., and Otis, D.L. 1982. Capture–recapture and removal methods for sampling closed populations. Los Alamos National Laboratory LA-8787-NERP, Los Alamos, N.M., USA.

Whiteway, S.L., Biron, P.M., Zimmerman, A., Venter, O., and Grant, J.W.A. 2010. Do in-stream restoration structures enhance salmonid abundance? a meta-analysis. Can. J. Fish. Aquat. Sci. **67**(5): 831–841. doi:10.1139/F10-021.

Young, M.K., Haire, D., and Bozek, M.A. 1994. The effect and extent of railroad tie drives in streams of southeastern Wyoming. West. J. Appl. For. **9**(4): 125–130.

Effort and potential efficiencies for aquatic non-native species early detection

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Abstract: Our objective was to determine the effort required for high-probability early detection of non-native zooplankton, benthic invertebrates, and fish using Duluth–Superior Harbor — a Great Lakes port under intense non-native species introduction pressure — as a case study. Initially, we allocated samples using a spatially balanced random design. We then resampled the harbor, but allocated samples to a few targeted areas. We detected 21 non-native invertebrate and 10 non-native fish species; however, many rare zooplankton and benthic invertebrates were likely missed. The two designs did not have significantly different species accumulation curves, but the targeted area design samples had higher species richness and detected non-native species with a significantly higher probability. It was possible to reduce the effort required to detect established non-native species. In contrast, the effort required to detect an ultra-rare, newly arrived species remained large. Based on statistical estimation theory, the effort required to detect 95% or more of species present could exceed enumerating 750 zooplankton samples (~500 000 individuals, ~90 species), 150 benthic invertebrate samples (~100 000 individuals, ~250 species), and 100 fish samples (~75 000 individuals, ~40 species).

Résumé : En menant une étude de cas dans le port de Duluth–Supérieur — un port des Grands Lacs qui subit une pression intense d'introduction d'espèces non indigènes — notre objectif est de déterminer les efforts requis pour obtenir une forte probabilité de détection du zooplancton, des invertébrés benthiques et des poissons non indigènes. Au départ, nous avons réparti les prélèvements selon un plan d'échantillonnage aléatoire spatialement équilibré. Nous avons ensuite échantillonné de nouveau le port, mais fait les prélèvements dans un nombre limité de zones stratégiques. Nous avons détecté 21 espèces d'invertébrés et 10 espèces de poissons non indigènes; cependant, nous avons vraisemblablement manqué plusieurs espèces de zooplancton et d'invertébrés benthiques rares. Les deux plans produisent des courbes d'accumulation d'espèces qui ne sont pas significativement différentes, mais le plan qui cible certaines zones particulières donne des richesses spécifiques plus élevées et détecte les espèces non indigènes avec une probabilité significativement plus grande. En revanche, l'effort nécessaire pour détecter les espèces ultrarares et nouvellement arrivées reste élevé. D'après la théorie de l'estimation statistique, l'effort requis pour détecter 95 % ou plus des espèces présentes pourrait dépasser une énumération de 750 échantillons de zooplancton (~500 000 individus, ~90 espèces), 150 échantillons d'invertébrés benthiques (~100 000 individus, ~250 espèces) et 100 échantillons de poissons (~75 000 individus, ~40 espèces).

[Traduit par la Rédaction]

Introduction

Biological invasion by aquatic non-native species is a problem that affects water bodies, both marine and freshwater, worldwide (Mills et al. 1994; Ruiz et al. 1997; Lodge et al. 1998). Beyond prevention, the principal strategies for managing non-native species are eradication and control (Hulme 2006; Lodge et al. 2006). Eradication and control are more likely to succeed if an introduced species is detected early, when its abundance is low and its spatial distribution restricted (Myers et al. 2000). These same distributional properties, however, hinder early detection. Monitoring areas vulnerable to invasion (Buchan and Padilla 2000; Loo et al. 2007; Vander Zanden and Olden 2008) or for organisms likely to invade (Kolar and Lodge 2002) may improve early detection. Sampling designs to detect non-native species, however, are rarely statistically based; results are often difficult to interpret because only positive detections are reported and surveys are qualitative (presence–absence), not quantitative (abundance-based; Hulme 2006). Thus, the effort required to detect a recently introduced, unspecified non-native species in a vulnerable area with significant statistical power (i.e., towards a generalized early detection model) is generally unknown (for an example limited to a single aquatic species, see Harvey et al. 2009).

Designing an early detection survey presents considerations common to all biodiversity sampling: biases from the survey design and collection methods (e.g., Fitzpatrick et al.

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2009), spatial- and temporal-scale effects on species distribution and abundance (e.g., Magnuson et al. 1994), power to detect presence and estimate abundance of organisms (e.g., Harvey et al. 2009), and effort and cost associated with collection and taxonomic classification (e.g., Lodge et al. 2006; Rew et al. 2006). Generally, the best design for rare species detection is to spread effort by sampling less frequently at more sites (MacKenzie and Royle 2005), though other spatial strategies such as sampling targeted transects along potential pathways may improve non-native species detection (Rew et al. 2006). Alternatively, strategies that alter sample allocation to maximize the number of non-native species detected in a survey can be developed by exploiting differences in species composition among sampling equipment and habitats (Trebitz et al. 2009). Non-native species surveys require time-intensive species-level identifications, yet they generally have small budgets (Steinberg et al. 2007), so identifying potential efficiencies is important to improve survey success.

Species accumulation theory describes the probability of detecting species and can be used to estimate the probability that some species were missed. Species accumulation theory generally has not been applied to the problem of aquatic non-native species detection, although these methods are essential for assessing the efficiency of different biodiversity sampling approaches (Gotelli and Colwell 2001). Generally, there is a strong, positive correlation between the number of individuals sampled and species richness, particularly when only a relatively few individuals have been sampled. This is known as the rarefaction effect (Palmer et al. 2008) and is illustrated by a species accumulation curve (i.e., the plot of the cumulative number of species captured in the order in which the samples were acquired). Species richness increases rapidly early in the sample effort as common taxa are acquired and progressively slows as increasingly rare species are acquired; thus, the curve is asymptotic (Bunge and Fitzpatrick 1993). In the context of species accumulation theory, the problem of detecting a newly introduced species is akin to that of detecting a rare one. The likelihood of detecting a rare species increases with increasing effort and becomes high only after substantial, perhaps unprecedented, sampling effort. To be confident that a species has not been missed, observed richness should be near or at the theoretical asymptote of the species accumulation curve. The initial species accumulation rate depends on the spatial heterogeneity in an assemblage's distribution (Gotelli and Colwell 2001). Efficiencies might be gained early in the survey by exploiting this heterogeneity.

Our principal objective was to determine the effort required for high-probability early detection of non-native zooplankton, benthic invertebrates, and fish using Duluth–Superior Harbor, an international shipping port and non-native species invasion "hotspot," as a case study. In general, we used an "oversampling" strategy, exerting as much effort as feasible in an attempt to detect all or nearly all species present. A secondary objective was to compare species detection among slightly different survey designs. In essence, we asked whether an alternative design that limited sampling space based on environmental heterogeneity would change non-native species detection power and efficiency. To compare designs, we used species accumulation theory to estimate species richness, characterize species accumulation, and estimate the effort required for high-probability early detection (i.e., the effort required to detect 95% or 100% of the estimated species richness) for each taxonomic group. We also estimated detection probabilities for the actual non-native species we detected. They ranged from common and widespread to rare and localized, which is analogous to a non-native species at an initial invasion stage.

Materials and methods

Site description

The Duluth–Superior Harbor, located adjacent to Lake Superior at the mouth of the St. Louis River, receives transoceanic vessels from around the globe by way of the St. Lawrence Seaway and is the Great Lakes' largest commercial port. The harbor is one of four aquatic species introduction hotspots, regions with a large number of first discoveries of non-native species, that occur in the Great Lakes; the other three include the Lake Superior – Lake Huron corridor, the Lake Huron – Lake Erie corridor (including Lake St. Clair), and the Lake Erie – Lake Ontario corridor (Grigorovich et al. 2003*a*, 2003*b*). The most common vector for introduction has been ballast water discharge from commercial vessels; however, recreational boating, fishing, and aquarium release are also vectors (Grigorovich et al. 2003*b*; Holeck et al. 2004; Ricciardi 2006). At least 22 fishes and 16 aquatic invertebrates, largely from Europe and the Ponto Caspian region, pose a high risk of invading the Great Lakes in the near future (Kolar and Lodge 2002; Grigorovich et al. 2003*b*).

Our study region was the lower 35 km, from the river mouth to ca. 3 km below the first dam (Fond du Lac dam), which is a barrier to upstream movement by aquatic organisms (Fig. 1). This area encompasses a 48.6 km^2 river–wetland complex that is broadly characterized by a transition from a relatively undisturbed riparian zone and watershed in the upper reaches to an urbanized floodplain and shoreline about the Duluth–Superior Harbor. Available habitat includes natural and dredged river channels, embayments, shallow, vegetated areas, sand and mud flats, fringing wetlands, and ca. 75 km of armored shoreline (e.g., rip rap), retaining walls, and piers. Periodic seiches reverse the river flow direction up to Fond du Lac dam (Stortz and Sydor 1980), potentially facilitating upriver transport of planktonic non-native species.

Sampling approach

The survey's purpose was primarily to determine species present within targeted taxonomic group (i.e., a multispecies approach) and secondarily to estimate relative occurrence of all aquatic species, native or not, by identifying and tallying each species captured. Initially, we allocated samples across the harbor using a spatially balanced random survey to sample zooplankton, benthic invertebrates, and fish. We then resampled the harbor for benthic invertebrates and fish, maintaining a similar spatial extent but allocating samples over a limited space by targeting a few areas. Finally, we resampled a restricted number of targeted areas for fish by choosing only those areas where earlier sampling yielded high species richness or rare species. We obtained about equal numbers of samples under each design (Table 1). To reduce seasonal variability in species occurrence, field sampling directed towards a taxonomic group was limited to a four-week period between late July and early September.

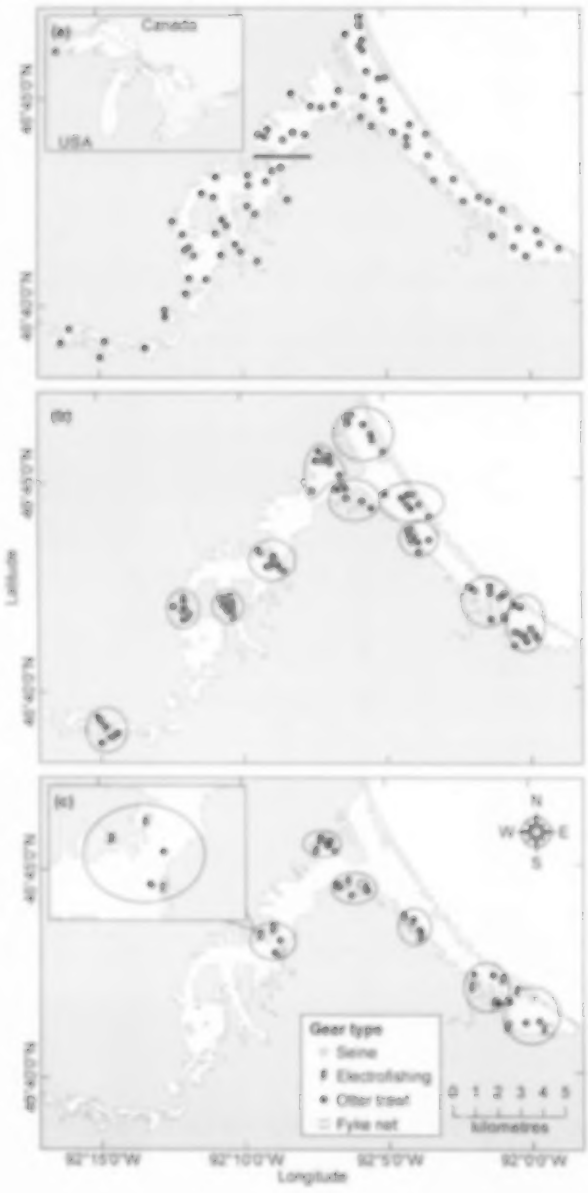


Fig. 1. Sampling station sites by design for (*a*) random site design (SBS), (*b*) targeted area design (SCS), and (*c*) spatially restricted design (rSCS). For the SBS design (*a*), the benthic invertebrates (petite PONAR grab only) and fish (gear allocated by depth) shared station locations. Zooplankton were sampled in the lower harbor only (downriver of the thick line) in 2005; stations are not shown but were dispersed in a similar manner. For SCS design (*b*), each targeted area ($n = 11$) is outlined with a thin line. Each dot within the targeted area represents a sample for benthic invertebrates (petite PONAR grab, benthic sled, sweep net, and Hester–Dendy sampler) or fish (electrofishing, fyke net, and otter trawl). For the rSCS design (*c*), each targeted area ($n = 6$) is outlined as in (*b*). Each fishing gear (except seine) was deployed at three locations within a targeted area; one location was sampled three different times (see inset).

By re-allocating samples within a shallow aquatic ecosystem that has a diversity of habitats, a slightly different mix of habitats is sampled under each design, requiring a different mix of sampling gears. Thus, sample allocation, habitat, and sampling gear are inherently confounded. This study was a first step toward addressing effort and potential efficiencies through the overall performance of some common survey design approaches; controlling for habitat- or gear-specific effects was outside the scope of these comparisons. We discuss habitat and sampling gear effects elsewhere (Trebitz et al. 2009, 2010; Peterson et al. 2011).

Random site design

A generalized random tessellation stratified survey design was used to provide an unbiased selection of sites dispersed throughout the Duluth–Superior Harbor (Stevens and Olsen 1999, 2004). This style of random survey design has come to be known as spatially balanced sampling (SBS; in the terminology of Theobald et al. 2007) because it uses reverse hierarchical ordering of selected sites to achieve spatial balance across the study area. In 2005, we conducted a pilot SBS design in the lower river for benthic invertebrates and zooplankton (Fig. 1*a*); benthic invertebrate data were used to establish sampling protocols and are not presented here. This was the only year in which we sampled zooplankton. In 2006, we sampled benthic invertebrates and fish at a set of 70+ common sites (Fig. 1*a*). We sampled zooplankton by net or pump based on depth, benthos by petite PONAR grab because it can be deployed in most locations, and fish by the gear most appropriate for the habitat and depth of the site (Table 1).

Targeted area design

We targeted a set of areas based on an array of attributes meant to characterize invasion vulnerability (Fig. 1*b*). We began with a geographic information system (GIS) description (1 ha cells) using a set of variables that we could access or model representing a habitat attribute, some aspect of a potential introduction vector, or both (Appendix A, Table A1). We used a principal components analysis (PCA) and an associated *k*-means cluster analysis to ordinate the cells and thereby identify a set of different qualities of environment, analogous to the approach described by Danz et al. (2005). The analysis divided the study area into six, mostly homogenous regions that were principally arrayed along the longitudinal axis of the river. To both cover the study region's extent and distinguish different representative combinations of environmental attributes, we selected 11 sampling areas based on these clusters (Fig. 1*b*). Instead of sampling a single site within each area, we sampled each with an array of gears to cover the range of microhabitats (e.g., vegetation beds) found within it. The size of each targeted area varied; effort was spread to include all attributes (clusters) present. We refer to this design as stratified cluster sampling (SCS; terminology of Theobald et al. 2007). We sampled benthos by sweep net, Hester–Dendy colonization sampler, benthic sled, and petite PONAR grab (generally, two PONAR samples from the SBS design fell in a targeted area and were included in analyses of either design). We sampled fish by electrofishing, fyke net, and otter trawl (Table 1).

Table 1. Number of stations and sample sizes for the random site (SBS), targeted area (SCS), and spatially restricted (rSCS) designs.

Design	Group	Year	Stations	Total sample size (by gear)	Samples per station
SBS	Zooplankton	2005	60	60 (19 N, 41 D)	1 sample
	Benthic invertebrates	2006	77	77 (77 P)	1 P
	Fish	2006	72	95 (39 EF, 25 F, 31 T)	1 sample
SCS	Benthic invertebrates	2006	11	64 (22 P, 22 B, 11 H-D, 9 S)	2 P, 2 B, 1 H-D, 1 S
	Fish	2006	11	64 (22 EF, 22 F, 20 T)	2 EF, 2 F, 2 T
rSCS	Fish	2007	6	108 (30 EF, 30 F, 30 T, 18 SE)	5 EF, 5 F, 5 T, 3 SE

Note: Sample size for each gear is given in parentheses by design and taxa: P, PONAR grab; B, benthic sled; H-D, Hester–Dendy sampler; S, sweep net; N, net tow; D, diaphragm pump; EF, electrofish; F, fyke net; T, otter trawl; SE, beach seine.

Spatially restricted design

A third survey design that only applied to fish used the original SCS base but narrowed the number of targeted areas from 11 down to a set of six that met one or more criteria based on the 2006 survey — close proximity to active shipping facilities, relatively high fish species diversity, and presence of rare species (i.e., present at one–two target stations in 2006; Fig. 1*c*). We intended to compare results using this restricted SCS design (rSCS) with the SCS and SBS designs to determine if prior knowledge of species distributions could improve species accumulation with less comprehensive spatial coverage but similar total effort. Equal effort was allocated to each sampling gear (electrofishing, fyke net, otter trawl), and each gear was deployed repeatedly within an area, allocating samples across both time and space (Fig. 1*c*). Because three of six areas overlapped with a Wisconsin Department of Natural Resources (WI DNR) biweekly beach seine index site, we added biweekly seine sampling at the remaining three stations to obtain sufficient data for inclusion of this gear in analyses.

Field and laboratory methods

Zooplankton

We sampled zooplankton by diaphragm pump at all sites <4 m deep and at a few sites 4–6 m deep (1000 L samples; inflow fitted with a coarse debris filter; outflow pumped through a 63 µm mesh net). We sampled by vertical net tow (63 µm mesh, 30 cm diameter) at most sites 4–6 m deep and all sites >6 m deep. We conducted multiple tows at a station to obtain a sample volume similar to that of the pump samples (total distance of 15–20 m, or 850–1130 L samples assuming 80% efficiency). Samples were preserved in 10% formalin. In the lab, samples were rinsed, diluted, and split. Replicate counts were obtained at a higher split volume, with two additional counts at one-half and one-quarter volumes, respectively, of the initial split. Zooplankton were identified to species (n = 31) or genus (n = 7).

Benthic invertebrates

We used four different gears to sample benthic invertebrates. We used a petite PONAR grab (0.024 m^2) to collect surface and subsurface sediment in water 0.8–13.4 m deep. We used a pole-mounted sweep net (310 mm × 170 mm D-shaped frame; 660 µm mesh bag) to sample submerged aquatic vegetation (SAV) where present (at two stations, no SAV was found). Standard samples required 3–5 min of effort per station, working the net through multiple SAV beds. We deployed Hester–Dendy samplers in late July for a standard 36 days. We used a modified D-shaped bottom sled to sample surface sediment (305 mm wide × 305 mm tall; 1000 µm mesh bag). Two standard 50 m tows were taken at each target station at depths of 1.3–9.6 m.

In the lab, formalin-preserved samples were rinsed, and organisms were picked, counted, and stored in 70% ethanol. All organisms were identified to the best taxonomic resolution possible using current keys and literature; specimens of questionable or unknown identification were sent to an additional taxonomic expert. Identification was generally to species (n = 102) or genus (n = 97). The best possible resolution for some taxa, however, was only to family (n = 21) or coarser (n = 6). All non-native species were identified to species (at least as adults). For simplicity, we refer to all unique taxonomic identifications as "species" (226 total).

Some poorly preserved specimens were incompletely identified, and early life stages of some taxa could not be identified to the same classification as later life stages. To resolve these taxonomic ambiguities, we assigned poorly classified specimens and early life stages to whichever well-classified taxon (generally, species) was most common at that sample or across samples (Cuffney et al. 2007). Although this distorts relative abundance because the most common taxa gain individuals, it conserves taxa richness better than the alternatives of distributing proportionally among potential taxa (inflating sample-level richness) or assigning it to the coarsest classification (discarding taxonomic information).

Fish

Fishing gear was chosen based on site depth. At sites 0–1 m deep, we set paired fyke nets, oriented lead-to-lead and parallel to shore (24 h set, 48 mm mesh, 7.5 m leads, 3 m wings). At sites 1–2 m deep, we electrofished for 10 min (5.3 m boom-shock Jon boat; Smith-Root 5.0 GPP electrofisher, 120 Hz DC). At sites >2 m deep, we sampled using a semi-balloon otter trawl (5-min tows at 3 $km \cdot h^{-1}$; 4.9 m head rope, 3.8 cm stretch mesh body, 31.8 mm stretch mesh cod end with 3.13 mm stretch mesh liner). For 2007, we pulled a 15.2 m bag seine parallel to shore for 30.5 m, following WI DNR procedures (1.8 m tall, 0.48 cm mesh). Sampling was conducted in August–September to allow juvenile fish to recruit to the gears and to complete sampling before aquatic vegetation senesced. At SBS design stations, the sample was centered at the coordinates selected regardless of microhabitat type. At SCS and rSCS design stations, gears were deployed to sample the range of microhabitats present.

Identifications were to species except young-of-year native sunfishes (pumpkinseed (*Lepomis gibbosus*) and bluegill (*Lepomis macrochirus*)) and bullheads (black (*Ameirus*

melas) and brown (*A. nebulosis*)), which were to genera because they cannot be reliably distinguished at this stage. Specimens that were not positively identified in the field were photographed or preserved for laboratory identification.

Analytical methods

We used species accumulation theory to estimate species richness and characterize species accumulation for the various survey designs, as well as to quantify the effort required for high-probability non-native species detection. We generated species accumulation curves by taxonomic groups (e.g., fish) using the sample-based rarefaction curve, which is the statistical expression of the species accumulation curve derived from the random re-ordering of its constituent sampling events (Chiarucci et al. 2008). This formulation takes into account natural patchiness in distributions (Gotelli and Colwell 2001). We scaled sampled-based rarefaction curves to the number of individuals encountered to account for systematic efficiency differences among sampling events (Gotelli and Colwell 2001), but we also scaled them to samples because this is a natural unit of effort. Species counts were not adjusted for volumetric subsampling (when it occurred) because multiplying by volume correction factors would artificially inflate the number of individuals relative to the number of species. We report the 95% confidence intervals (CIs), as well as the number of samples by which at least 50% of S_{est} ($S50\%$) were sampled (based on the rarefaction curve, assuming random reordering of samples), as a proxy for the initial species accumulation rate. The faster the rate at which the curve rises, the more species-rich the data set will be (i.e., there are more species per individuals encountered).

In theory, the total number of species is equal to the asymptote of the species accumulation curve; the empirical curve, however, may lack a well-defined asymptote. To estimate total species richness (S_{est}) independent of the curve, we used a nonparametric, sample-based estimator (Chao 1987; Chao et al. 2009): $S_{est} = S_{obs} + (1 - 1/t)Q_1^2/(2Q_2)$, where S_{obs} is the number of species in the sample set, t is the number of samples, and Q_1 and Q_2 are the number of "uniques" (species that occur in only a single sample) and "duplicates" (species that occur in only two samples), respectively. The estimator provides a lower bound (Chao 1987), which, however, breaks down when $Q_2 = 0$; it has a modified bias-corrected form (Chao 2005): $S_{est\ (bc)} = S_{obs} + (1 - 1/t)Q_1(Q_1 - 1)/(2(Q_2 + 1))$. We calculated species accumulation curves (based on the algorithm and CIs developed by Mao et al. (2005)) and S_{est} values ("Chao2") using the EstimateS v8.20 software (Colwell 2006). Following Colwell (2006), we report bias-corrected S_{est} values unless there was substantial heterogeneity among species in their probabilities of detection (incidence-based coverage estimator of species richness coefficient of variation (CV) > 0.5), in which case we report the uncorrected S_{est} value.

To quantify the effort required for high-probability non-native species detection, we estimated the number of samples required to obtain a near-total (95%) or total (100%) census of the zooplankton, benthic invertebrate, and fish species present. We used the nonparametric method proposed by Chao et al. (2009), which calculates the number of samples required to detect some proportion of the asymptotic sample-based species richness estimator S_{est}.

We estimated sample-based non-native benthic invertebrate and fish species detection probabilities (i.e., the probability that a species occurs in a sample) on a design basis. We defined non-native species as those that are not endemic to either the St. Louis River or the Lake Superior basin. We assumed that a species could be detected (i.e., captured and identified) if it was available to the sampling gear, although detection probabilities likely are influenced by gear type. Detection probabilities for non-native species were compared by taxonomic group using a nonparametric, repeated-measures test (Friedman two-way analysis of variance; Systat 11, Systat Software Inc., Chicago, Illinois). We examined species overlap among designs using the Morisita–Horn index (EstimateS v8.20), which is not sensitive to sample size because the index is most influenced by abundant species (Wolda 1981). It measures the similarity of species' abundance with values ranging from 0 (no shared species) to about 1.

Finally, we quantified the effort required for high confidence detection of present non-native species, which we define as a detection probability ≥95%, using a nonparametric method to estimate the cumulative probability of detecting identified non-native benthic invertebrates and fish. We generated 1000 randomized sequences of all the samples in either the SBS design (benthic invertebrates) or the SBS and SCS designs (fish). The first occurrence of each non-native species in each sample sequence was identified and the station order numbers were tallied so that we could plot the cumulative distribution of first occurrences across sample sequences.

Results

Zooplankton

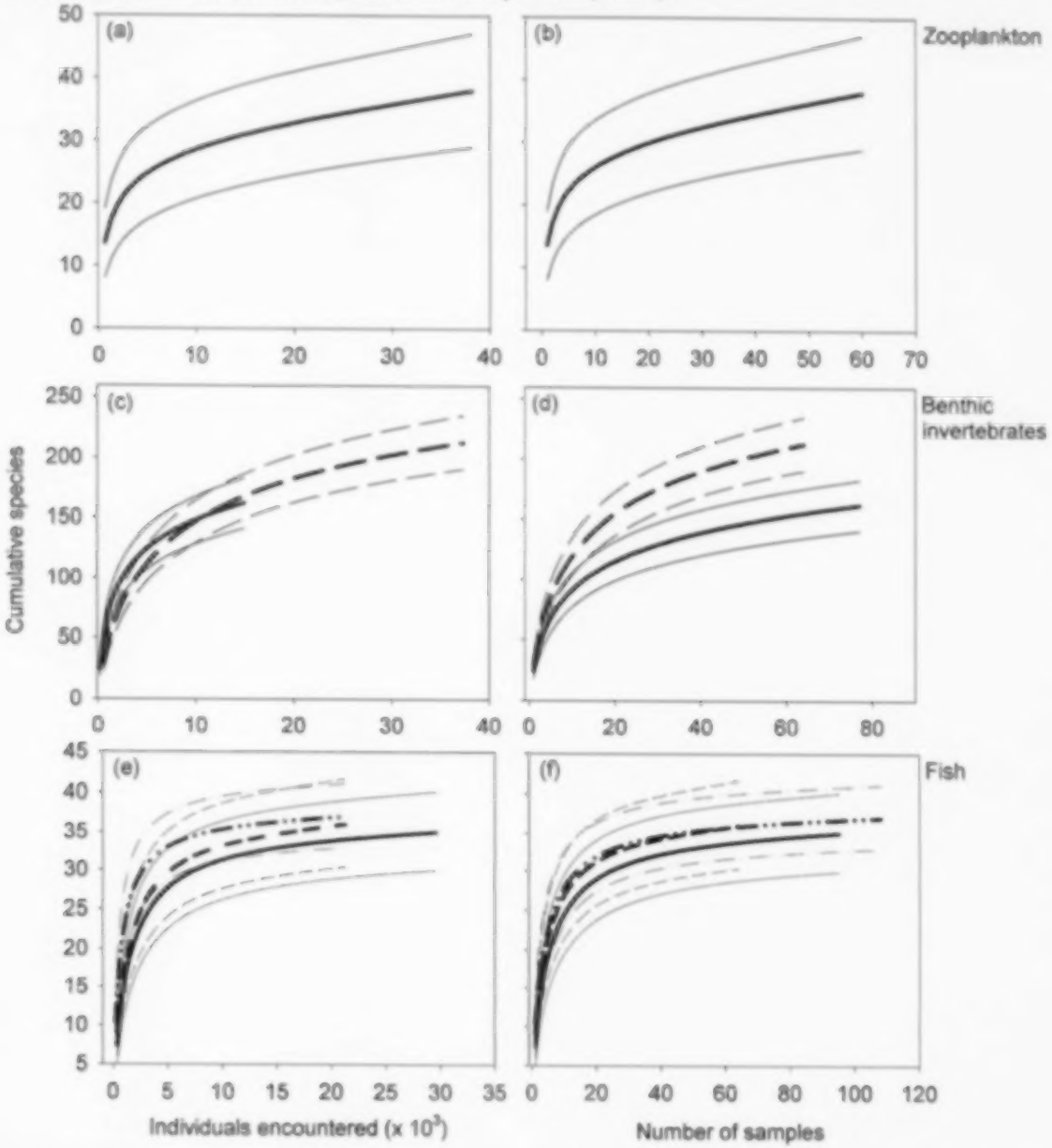
We detected (captured and identified) 38 zooplankton over 60 samples in the random site design (SBS). The zooplankton rarefaction curve did not reach an asymptote, indicating that the zooplankton assemblage was not fully sampled (Figs. 2*a*, 2*b*). The total species estimate (S_{est} = 88) was much greater than our actual species tally (S_{obs}) and had a broad CI (Table 2). We estimated that a total of 776 samples would be required to detect 95% of S_{est} ($S95\%$; ca. 500 000 individuals) and nearly 2000 samples to detect 100% of S_{est} ($S100\%$, >1 000 000 individuals; Table 2). Two non-native zooplankton species previously detected in the harbor, the calanoid copepod *Eurytemora affinis* (97% of samples) and the cladoceran *Eubosmina coregoni* (27% of samples), were common. The non-native cladoceran *Bythotrephes longimanus*, which has been observed in the harbor (Grigorovich et al. 2003*b*), was not detected.

Benthic invertebrates

We detected 162 benthic invertebrate species in the SBS design, whereas 213 species were detected in the targeted area design (SCS; Table 2). Accordingly, the S_{est} was higher in the SCS design (259) than in the SBS design (205). For both designs, the rarefaction curve did not reach an asymptote (Fig. 2*c*), and thus S_{est} was much higher than S_{obs} and had a broad CI (Table 2).

The SCS design acquired benthic invertebrate species at a similar rate to the SBS design (Fig. 2*c*). Acquiring half of S_{est} ($S50\%$) using the SCS design would require a similar sample

Fig. 2. Sample-based rarefaction curve for (*a*, *b*) zooplankton species, (*c*, *d*) benthic macroinvertebrates, and (*e*, *f*) fish. Each plot shows the mean (thick line) with the associated 95% confidence intervals (thin lines) by survey design (SBS, random site design, solid line; SCS, targeted area design, dashed line; rSCS, spatially restricted design, dashed–dotted line). Sample-based rarefaction curves are scaled by the number of individuals encountered (right side) or by the number of samples taken (left side).



burden to that of the SBS design but would require enumerating three times as many individuals (ca. 7600 and 2700, respectively, based on the mean individuals per sample), reflecting the higher species tally and greater number of individuals encountered (Table 2).

Under the SBS design, 208 and 671 samples are required to detect *S*95% and *S*100% (ca. 130 000 individuals), respectively. Under the SCS design, a smaller relative effort (150 and 497 samples, respectively) is required, but the number of individuals required to enumerate is greater (ca. 90 000 or 290 000, respectively), in part due to the greater S_{mn}. Scaling the rarefaction curves by samples (Fig. 2*d*), the SCS design appears to perform significantly better than the SBS design because the petite PONAR grab samples (the only kind taken at SBS design stations) contained fewer organisms per sample than the other gears.

Table 2. Sample sizes and performance measures for the various taxonomic groups and survey designs.

Design	No. of samples	No. of individuals	Q_1	Q_2	S_{obs}	S_{est} (95% CI)	No. of samples for *S*50%	Additional samples		Additional individuals	
								*S*100%	*S*95%	*S*100%	*S*95%
Zooplankton											
SBS	60	38 168	10	1	38	88 (46–354)	36	1922	716	1 222 648	455 471
Benthic invertebrates											
SBS	77	14 901	36	15	162	205 (181–262)	14	594	131	114 951	25 351
SCS	64	37 312	49	24	213	259 (236–308)	13	433	86	252 439	50 138
Fish											
SBS	95	29 590	3	1	35	36 (35–50)	5	519	115	161 655	35 819
SCS	64	21 234	4	1	36	44 (37–101)	5	552	165	183 143	54 744
rSCS	108	21 141	2	1	37	38 (37–45)	4	291	2	56 963	392

Note: SBS, random site; SCS, targeted area; rSCS, spatially restricted. Survey data include the sample number, individuals tallied (excludes juveniles not identified to species), "uniques" (species captured in one sample; Q1), "duplicates" (species captured in two samples; Q2), and observed species richness (S_{obs}). Performance measures include the estimated sample-based species richness (S_{est}) with associated confidence interval (CI), as well as the number of samples required to sample half S_{est} (*S*50%; based on the associated sample-based rarefaction curve), and the effort required to sample 95% (*S*95%) or 100% (*S*100%) of S_{est} based on Chao et al. (2009), expressed as both the number of samples and the number of individuals (number of samples multiplied by the mean number of individuals per sample).

The detection probabilities for non-native benthic invertebrates were significantly different among the SBS and SCS designs (Friedman two-way analysis of variance (ANOVA), $p = 0.001$; Table 3). The SBS design detected fewer non-native benthic species than the SCS design (16 versus 19, respectively). Eight non-native benthic invertebrates were new detection records in the St. Louis River during this study, two of which were only detected in the SCS design: the humpback peaclam (*Pisidium supinum*) and the cladoceran *Daphnia lumholtzi* (Table 3). The Morisita–Horn index value, 0.69, indicated some overlap; 147 species were shared among designs (Table 4). The SBS design detected 162 species, indicating that the SCS design captured most native species detected in the SBS design, as well as more non-native species (Table 3).

Fish

We detected 35 fish species in the SBS design, 36 in the SCS design, and 37 in the restricted targeted area design (rSCS; Table 2). The rarefaction curve for the SBS design did approach an asymptote (Figs. 2*e*, 2*f*), and thus the S_{est} (36) was similar to the S_{obs}. The rarefaction curve for the SCS design reached a higher asymptote, resulting in a greater S_{est} (44) than for the SBS design, but with a broad CI. The S_{est} for the rSCS design was intermediate between S_{est} values for the other two designs (Table 2).

The rarefaction curves for fish were similar among the SBS and SCS designs (Fig. 2*e*). The rSCS design initially acquired species significantly faster than the SBS design (i.e., the mean curve for the rSCS design was above the upper 95% CI of the SBS design curve) but was similar to the SCS design. Under either the SBS or SCS design, the *S*50% could be acquired in five stations (Table 2), or about 1500 individuals, whereas under the rSCS design, it would be acquired within four stations, or about 800 individuals.

The effort exerted in the SBS design was about half that required to detect *S*95%; 614 samples would be required to detect *S*100% (ca. 190 000 individuals; Table 2). Under the SCS design, 229 and 616 samples would be required to detect *S*95% and *S*100% (ca. 204 000 individuals), respectively. The greater effort required compared with the SBS design reflects the higher S_{est} of the SCS design. The effort exerted in the rSCS design was sufficient to detect *S*95%. A nearly four-fold increase in effort (399 samples) would be required to detect *S*100%, though this is equivalent to many fewer fish than in the other designs (ca. 78 000 individuals).

The SBS design detected fewer non-native fish species than the SCS and rSCS designs (nine versus 10). The detection probability for non-native fishes was significantly different between the SCS and SBS designs (Friedman two-way ANOVA, $p = 0.011$; Table 5). The detection probabilities between the SCS and rSCS designs were not significantly different ($p = 0.058$); however, except for alewife (*Alosa pseudoharengus*) and white perch (*Morone americana*), the detection probabilities were higher for the rSCS design. The Morisita–Horn index revealed a high proportion of shared species among all three designs; however, the rSCS design was more dissimilar to the SBS and SCS designs than the SBS and SCS designs were to each other (Table 4). The 10 species detected include all non-native fishes known to reside in the study area.

Non-native species detection

Cumulative detection probability functions generated by randomization reflect each non-native species' relative occurrence (Fig. 3). For species that were widely distributed and abundant such as zebra mussel (*Dreissena polymorpha*), round goby (*Neogobius melanostomus*), and Eurasian ruffe (*Gymnocephalus cernuus*), a >95% detection probability was achieved within 10 samples. For non-native species that were quite rare such as the Asian clam (*Corbicula fluminea*), greater European peaclam (*Pisidium amnicum*), and three-spine stickleback (*Gasterosteus aculeatus*), a >95% detection probability was achieved within ca. 75 (benthic invertebrates) to 100 stations (stickleback). At greater than 40 samples, the probability was >50% that these species would be detected. For non-native benthic invertebrates, the functions are distributed broadly between the two extremes, with about one-third of the species (seven) sufficiently rare to require a large sample burden for high-confidence detection (Fig. 3*a*). In contrast, for non-native fishes, the functions are more centrally distributed on the plot (Fig. 3*b*).

Table 3. Probability of detecting non-native benthic macroinvertebrate species (p; the proportion of samples in which a species was present) and the numbers of individuals tallied (count) in either the random site (SBS) or targeted area (SCS) designs, sorted from rare to common.

		p(detection)		Count	
Species	Common name	SBS	SCS	SBS	SCS
*Pisidium supinum**	Humpback peaclam	0.00	0.02	0	1
*Daphnia lumholtzi**	Water flea	0.00	0.02	0	1
Cordylophora caspia	Freshwater hydroid	0.00	0.02	0	1
Pisidium amnicum	Greater European peaclam	0.01	0.03	2	9
Corbicula fluminea	Asian clam	0.01	0.02	1	156
*Paranais frici**	Oligochaete	0.03	0.03	60	60
*Pristina acuminata**	Oligochaete	0.03	0.03	12	2
Ripistes parasita	Oligochaete	0.03	0.14	2	42
*Potamopyrgus antipodarum**	New Zealand mudsnail	0.03	0.13	11	55
*Dreissena bugensis**	Quagga mussel	0.03	0.16	2	45
Gammarus tigrinus	Amphipod	0.03	0.05	2	17
*Echinogammarus ischnus**	Amphipod	0.04	0.13	3	38
Valvata piscinalis	European valve snail	0.05	0.08	11	46
Pisidium moitessierianum	Pygmy peaclam	0.06	0.17	13	40
Potamothrix moldaviensis	Oligochaete	0.10	0.06	36	17
Gammarus fasciatus	Amphipod	0.17	0.31	155	255
Dreissena polymorpha	Zebra mussel	0.18	0.53	357	5 039
Potamothrix vejdovskyi	Oligochaete	0.19	0.17	154	110
*Pisidium henslowanum**	Henslow's peaclam	0.29	0.33	132	350
Dreissena spp. (juvenile)		0.40	0.75	127	82 598

Note: Asterisk (*) indicates new detection record for the St. Louis River.

Table 4. Morisita–Horn similarity index values and the number of shared species among designs for the various design contrasts (SBS, random site; SCS, targeted area; rSCS, spatially restricted).

Taxonomic group	Contrast	Shared species	Morisita–Horn
Benthic invertebrates	SBS × SCS	147	0.692
Fish	SBS × SCS	33	0.906
	SBS × rSCS	34	0.479
	SCS × rSCS	33	0.460

Table 5. Probability of detecting non-native fish species (p; the proportion of samples in which a species was present) in either the random site (SBS), targeted area (SCS), or spatially restricted (rSCS) designs, sorted from rare to common.

		p(detection)		
Species	Common name	SBS	SCS	rSCS
Gasterosteus aculeatus	Threespine stickleback	0.01	0.02	0.07
Aplodinotus grunniens	Freshwater drum	0.03	0.02	0.06
Alosa pseudoharengus	Alewife	0.03	0.05	0.01
Labidesthes sicculus	Brook silverside	0.00	0.08	0.15
Osmerus mordax	Rainbow smelt	0.09	0.11	0.18
Cyprinus carpio	Common carp	0.13	0.19	0.32
Proterorhinus marmoratus	Tubenose goby	0.16	0.25	0.30
Morone americana	White perch	0.23	0.34	0.11
Gymnocephalus cernuus	Eurasian ruffe	0.46	0.50	0.54
Neogobius melanostomus	Round goby	0.39	0.52	0.88

Discussion

Overall, 21 non-native aquatic invertebrate and 10 non-native fish species were detected. This list included nearly all previously known aquatic non-native species in the St. Louis River plus eight new non-native benthic invertebrates (Grigorovich et al. 2008; Trebitz et al. 2009, 2010). We attribute the detection success to taxonomic expertise and the exhaustive sampling effort; about 40 000 zooplankton, 52 000 benthic invertebrates, and 70 000 fish were tallied and identified in the course of the sampling reported here. The rarefaction curves, however, indicate that despite this intensive effort, many rare species, espe-

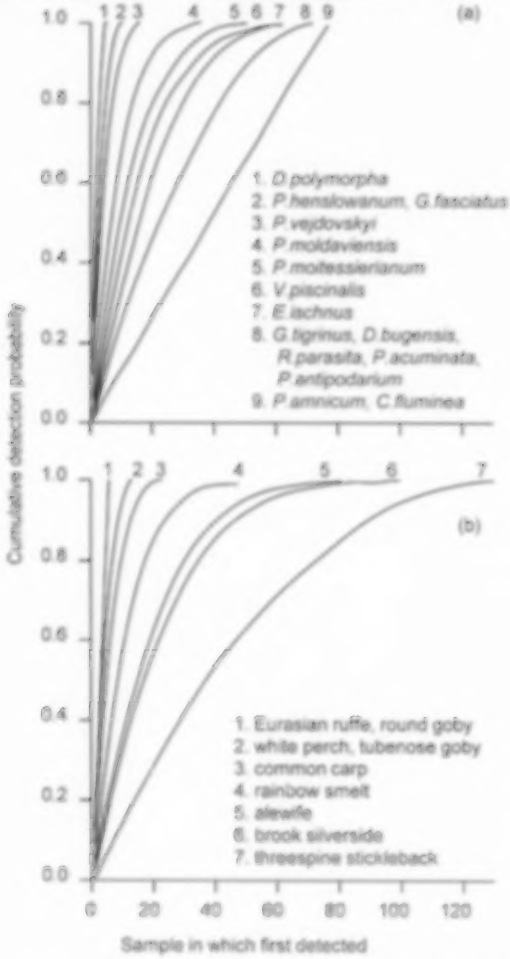


Fig. 3. Cumulative probability of detecting either (*a*) non-native benthic macroinvertebrate species in a sample based on Monte-Carlo randomization of the 2006 PONAR grab data or (*b*) non-native fish species in a sample based on Monte-Carlo randomization of the 2006 fish data (random site (SBS) and targeted area (SCS) stations pooled). Overlapping lines have been combined for clarity.

cially zooplankton and benthos, likely were not detected. One or more newly arrived, non-native species could be among those undetected and presumably very rare species.

The effort required to detect 95% ($S95\%$) or more ($S100\%$) of the total estimated zooplankton, benthic invertebrate, and fish species in the Duluth–Superior Harbor using any of the designs that we employed would be substantial. More than 75 samples are required to achieve a >95% probability of detection for rare (i.e., detected in only one sample), established non-native species. Comparison of the alternative designs with the random site design (SBS) demonstrates that neither species richness nor species accumulation rates were reduced by focusing effort in a few areas. Rather, the targeted area design (SCS) had higher species richness, sampled similar native species, and detected non-native species with a significantly higher probability than the SBS design. Compared with the SBS design, however, the effort required under the SCS design to detect similar proportions of the species pool (S_{est}) was only slightly reduced with respect to fish and was actually increased with respect to benthic invertebrates. We discuss these findings with regard to the sampling effort required for aquatic non-native species early detection and the role of survey design in achieving detection efficiencies.

Required sampling effort

The effort required for high-probability, early detection of aquatic non-native species is substantial. Because Lake Superior has fewer species than the lower Great Lakes (Grigorovich et al. 2003*b*) or marine ports, even greater effort than we expended is likely required in other regions highly vulnerable to invasion, but this remains to be demonstrated. Given the large effort required to sample the entire species pool ($S100\%$) and that non-native species survey programs operate under small and declining budgets (Steinberg et al. 2007), surveys may have to aim to detect 95% of the species pool (Chao et al. 2009). The associated risk is that some rare species, possibly a recently introduced non-native species, would remain undetected. Unfortunately, the extra effort required to detect the remaining 5% of species is daunting. In only the rSCS design did we deploy sufficient effort (108 samples, ca. 21 000 fish) to sample 95% of the total species pool, but to capture the remaining zero–eight fish species would require us to obtain an additional 291 samples or 57 000 fish.

Detecting 95% of all species is a potentially feasible design goal. For benthic invertebrates, in the best case (i.e., a SCS-like design), the effort required to achieve this goal in a single region or port could exceed enumerating 100 000 individuals, or collecting and processing more than 150 samples, assuming similar species richness (ca. 250 species). This is a much greater sample burden than required to obtain a 95% detection probability for those non-native species that were actually detected. The difference arises because the former analysis pertains to the theoretical species pool, which includes many species that were presumably not detected, whereas the latter pertains to those that were detected and are thus, on average, more abundant than those not detected. For zooplankton (ca. 90 species), the effort could exceed 500 000 individuals or 750 samples. For fish, the effort could exceed 75 000 individuals or 100 samples. This latter estimate is comparable with that for a temperate estuarine fish assemblage sampled by trawl (S_{obs} = 70): S_{est} = 90 and $S95\%$ = 97 008 in total (Chao et al. 2009) and consistent with the necessary effort to detect the most rare, established non-native fish captured in this study. For Great Lakes stream fishes, using random site selection, over 100 stream reaches must be sampled to detect 100% of S_{est} (23–58; Smith and Jones 2005). Fish also may be difficult to census because the species pool undergoes exchange with the lake; for example, some rare species are not riverine species (e.g., we captured young bloater and adult quillback, which use river habitat intermittently). A similar problem may be encountered with planktonic invertebrates.

Periodically constructing rarefaction curves while sampling can help gauge performance (e.g., Smith and Jones 2005) and thus potentially avoid excess effort, but this would be difficult for invertebrates because the laboratory species identification typically lags the field collection by many months.

Ideally, survey progress could be evaluated against past inventories. This study, however, produced the most definitive benthic invertebrate inventory of Duluth–Superior Harbor completed to date (Trebitz et al. 2010). Fish are well inventoried: about 60 species can be found in the study area, excluding non-native anadromous salmonids that are only present during spring (St. Louis River Citizens Action Committee 2002). We detected 42 species, implying that 31% of species were missed, and the upper 95% CIs from two of three designs were less than 60. Even this comparison, however, is problematic. About half of the species that we did not detect are lake migrants and thus may not have been in the river. Also, our sampling did not include some unique habitats adjacent to the Fond du Lac dam that likely support additional species.

Spatially restricted non-native species present a formidable problem for early detection because a species could establish a viable population prior to detection if concentrated in a small area. For example, the Asian clam was narrowly distributed at the warm outfall of a waste disposal plant (a targeted area), but its abundance was consistent with a reproducing population (156 specimens). Forty samples are required to reach a 50% detection probability for this species. In contrast, widespread but less abundant species are readily detected. For example, only 40 specimens of the pigmy pea-clam (*Pisidium moitessierianum*) were found across all SCS stations, but it had a 50% detection probability within 10 stations. Unfortunately, those species that are easiest to eradicate because of their restricted spatial distribution are the most difficult to detect.

Role of sampling design

For benthic invertebrates, the targeted area (SCS) design had higher species richness than the random site design (SBS), with a high proportion of species overlap (91%). Although the two designs acquired species at a similar rate, SCS design samples were significantly more likely to include non-native species. The total effort (sample collection and processing) actually increased under the SCS design, however, because far more individuals (as well as species) were captured. The benthic invertebrates were not distributed homogenously (Trebitz et al. 2010) and we attribute the improved performance of the SCS design to the fact that multiple gears were used, consistent with other benthic invertebrate survey design studies (Turner and Trexler 1997; Bartsch et al. 1998). The effects of the additional gears, however, are confounded with the effects of sampling the additional habitats associated with each gear.

Habitat plausibly did contribute to the higher species richness sampled in the SCS design. Ordination of the benthic invertebrate data from this study demonstrated that non-native and rare species richness was higher at shallow stations with substantial vegetation cover than at stations with bare substrate (Trebitz et al. 2009). Those gears that were used in vegetated habitats, however, did not contribute equally to the improved non-native species detection rates. Sampling vegetated habitats (sweep net, Hester–Dendy sampler) in the SCS design did not yield new non-native species, but did yield new native species and higher detection rates for certain non-native species, particularly non-native amphipods and dreissenid mussels (Trebitz et al. 2010). The sweep net samples species richness well in vegetated habitat (Turner and Trexler 1997) and thus would be expected to capture rare species. Plausibly, littoral areas are resistant to invasion, but this merits further investigation and there remains uncertainty about properties affecting invasion resistance in aquatic systems (Ruiz et al. 1997; Ricciardi 2001; Stachowicz et al. 2002).

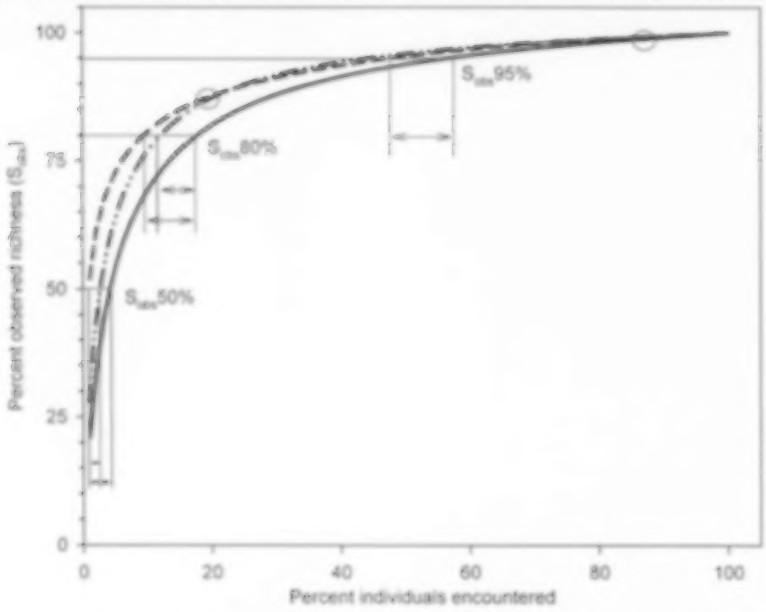
Gear-specific rarefaction effects likely also contributed to higher non-native species richness in the SCS design. The three additional non-native species captured in the targeted area design (the hydrozoan *Cordylophora caspia*, the cladoceran *Daphnia lumholtzi*, and the peaclam *Pisidium supinum*) were all captured in the benthic sled, which sampled about four times more individuals than the petite PONAR grab. Further, both *Daphnia lumholtzi* and *Pisidium supinum* were detected in the 2005 pilot study using a full-size PONAR grab, which also captured many more organisms than the petite PONAR grab.

For fish, species richness, overlap, and acquisition rates were similar among designs, indicating that there was not a loss of survey efficacy by targeting a few areas within the harbor. Further, there was a trend in species richness (S_{obs}), species accumulation rate, and non-native fish detection efficiencies from the SBS design (lowest) to the SCS design (intermediate) to the rSCS design (highest). Unlike benthic invertebrates, sampling gear bias likely had a small or no effect on the difference among designs because the effort allocated to each gear was similar under each. Rather, this trend implies that there is some efficiency gained by choosing stations to maximize their "information content." By distributing targeted areas across multiple environmental dimensions, only a few samples were allocated to noninformative station types. Generally, we obtained few fish when electrofishing shallow, sandy flats, whereas we obtained large numbers of a few well-established species such as Eurasian ruffe, white perch, and trout-perch when trawling in dredged navigation channels (Trebitz et al. 2009). The SCS design was likely more efficient than the SBS design because we did not repeatedly sample these species-poor habitats, whether low (e.g., open sand flats) or high (e.g., deep channel) density, that contributed little new information regarding non-native species presence. Also, by integrating sampling over various microhabitats within a small area, we were able to obtain more samples with fewer fish and thereby achieve a high probability of detection. These types of efficiencies are likely widespread; like other coastal aquatic ecosystems, the lower St. Louis River is a habitat mosaic wherein species are distributed patchily (Sheaves 2009).

Further efficiencies were achieved in the rSCS design by using prior data to identify stations known to be species-rich or to possess rare species. The overall effect was to reduce species-poor hauls, thereby increasing efficiency; there was a 35% reduction in the number of samples and 59% reduction in the number of fish required to detect the total estimated species pool ($S100\%$) compared with the SBS design. This large difference in abundance is reflected in the Morisita–Horn index. In theory, however, this strategy also increased the risk of failing to detect a new non-native species that invades a species-poor habitat.

A station-level rarefaction effect may also have contributed to survey performance. Compared with the SBS design, the SCS design increased sampling effort in structurally complex

Fig. 4. Sample-based fish species rarefaction curves for the random site (SBS; solid line) and spatially restricted (rSCS; dashed–dotted line) designs compared with the individual-based rarefaction curve for the random site design (dashed line), normalized for observed fish species richness (SBS = 35; SCS = 37) and individuals encountered (SBS = 29 590; rSCS = 21 141). The drop lines indicate various reference points with respect to the observed species pool. The circles indicate where the various rarefaction curves begin to overlap.



vegetated habitats — areas known to have high fish abundance (Smokorowski and Pratt 2007) and richness (Trebitz et al. 2009). The higher richness may simply be a rarefaction effect (i.e., there were more species simply because more fish were captured; Grenouillet et al. 2002). Similarly, the number of individuals sampled is a better predictor of fish species richness than habitat volume or complexity in some streams (Angermeier and Schlosser 1989). A station-level rarefaction effect, however, cannot account for the difference among designs because the rSCS design captured two more species than the SBS design, despite sampling 29% fewer fish.

The rSCS design plausibly capitalized on biological patterns by sampling a mix of heterogeneous habitat across a species composition gradient. In the St. Louis River, fish species richness, inclusive of non-native species richness, increases downstream, perhaps because it is an ecotone between the river and Lake Superior (Trebitz et al. 2009). The six areas sampled in the rSCS design were located in the lower half of the study region. Within each area, a mix of microhabitat, including river channel, mud or sand flat, fringing wetlands, and backwaters, each of which supports a slightly different assemblage, was sampled (Trebitz et al. 2009).

Although there are efficiencies associated with the targeted area designs, the overall effort to sample *S*100% is still very high (400–600 samples). This is because the greatest efficiencies can be achieved in the early part of the species accumulation curve, where natural patchiness in the assemblage can be exploited (e.g., by avoiding large, monotypic samples). This apparent paradox is illustrated by comparing the sample-based rarefaction curve, which includes sample heterogeneity, with the individual-based rarefaction curve, which assumes that individuals are distributed homogenously throughout samples, thereby ignoring any co-distribution among species (Gotelli and Colwell 2001). The difference between the sample-based and individual-based curves indicates the degree to which organisms are patchily distributed and, by extension, the degree to which efficiency could be gained if that patchiness was fully characterized and exploited (Fig. 4). Initially, common species are rapidly attained under any sampling effort and thus the *S*50% is similar. After this point, the two SBS-based rarefaction curves diverge and remain dissimilar through the middle portion of the curve, up to S_{obs}95%, by which time moderately abundant species are caught with relatively high probability. The two curves converge near the asymptote, at about S_{obs}98%, because the last few, very rare species are effectively distributed homogenously and thus appear with equal, low probability throughout the samples (e.g., *P. amnicum*). In comparison, the rSCS sample-based rarefaction curve lies intermediate between the two SBS-based curves, and the rSCS rarefaction curve merges with the SBS individual-based rarefaction curve at S_{obs}85%, rather than at S_{obs}98% (Fig. 4). Our interpretation of these pattern differences is that we were able to exploit heterogeneity in the assemblage to improve species detection in the rSCS design.

With respect to efficiency, our goal was to assess some different survey designs that cover space in different ways. Although we cannot fully ascribe underlying mechanisms responsible for observed differences, we have been successful at illustrating that performance differences exist. Our results demonstrate that we can modify survey design to increase species richness and improve detection of established species.

Improving species richness, however, may require greater overall sampling or taxonomic identification effort. Efficiency gains for detecting established non-native species can be significant and yet, paradoxically, do little to improve a full species census because of the rarefaction effect. Detecting the last few, ultra-rare species (i.e., at the early invasion stage) requires enormous effort relative to finding the more common, established species.

Early detection is a critical piece of a comprehensive non-native species control program (Lodge et al. 2006; Vander Zanden et al. 2010). Given the large effort required for early detection and the many potential points of introduction, early detection of every recently introduced species is not practically feasible. Vector control and prevention thus remain critical elements within a comprehensive invasive species strategy. Moreover, because the effort required to detect rare species is quite large relative to typical survey efforts, biological monitoring programs may not have the capacity to undertake such surveys (Vander Zanden et al. 2010). New diagnostic tools may facilitate rapid surveys over large spatial scales (Lodge et al. 2006). For example, environmental DNA screening is a promising method for detecting rare species because it is more sensitive than traditional sampling methods and is well suited to surveys across a large spatial scale (Jerde et al. 2011). Because DNA markers are species-specific, however, they are generally used to confirm specimen identity (e.g., Grigorovich et al. 2008; this study) or to detect target species in simple environmental samples (for review, see Darling and Blum 2007). Genetic tools for simultaneous screening for a wide variety of potential non-native species (i.e., the question of interest in this study) are still under development (Darling and Blum 2007).

Our findings have three major implications for designing early detection surveys. First, current exotic species monitoring efforts could likely be made more efficient. These efficiencies, however, are greatest at a level of effort that is well below that needed to capture ultra-rare species (i.e., the left-hand portion of the rarefaction curve). Thus, for these surveys to be able to detect new species before they have become established, it is necessary to both increase their efficiency through improved survey design and increase the level of sampling effort. Future research toward optimizing design based on habitat quality or gear performance can help guide further improvements (Smith and Jones 2008; Trebitz et al. 2009). The analytical approaches used in this study, species accumulation theory and cumulative detection probability, are key elements to assessing performance. Second, designs that limit spatial coverage based on existing ecological patterns can potentially achieve more efficient species detection. Imposing site criteria on sample allocation, however, risks the unintended effect of missing an exotic species that is attracted to a limited suitable habitat. Third, given that the effort required to detect 95% or more of the total estimated species using any design is substantial, the risk of not detecting a species must be recognized, and programs should be intentionally designed with defined levels of detection probability.

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References

Angermeier, P.L., and Schlosser, I.J. 1989. Species–area relationships for stream fishes. Ecology, **70**(5): 1450–1462. doi:10.2307/1938204.

Bartsch, L.A., Richardson, W.B., and Naimo, T.J. 1998. Sampling benthic macroinvertebrates in a large flood-plain river: considerations of study design, sample size, and cost. Environ. Monit. Assess. **52**(3): 425–439. doi:10.1023/A:1005802715051.

Buchan, L.A.J., and Padilla, D.K. 2000. Predicting the likelihood of Eurasian watermilfoil presence in lakes, a macrophyte monitoring tool. Ecol. Appl. **10**(5): 1442–1455. doi:10.1890/1051-0761(2000)010[1442:PTLOEW]2.0.CO;2.

Bunge, J., and Fitzpatrick, M. 1993. Estimating the number of species: a review. J. Am. Stat. Assoc. **88**(421): 364–373. doi:10.2307/2290733.

Chao, A. 1987. Estimating the population size for capture–recapture data with unequal catchability. Biometrics, **43**(4): 783–791. doi:10.2307/2531532. PMID:3427163.

Chao, A. 2005. Species estimation and applications. *In* Encyclopedia of statistical sciences. *Edited by* N. Balakrishnan, C.B. Read, and B. Vidakovic. Wiley, New York. pp. 7909–7916.

Chao, A., Colwell, R.K., Lin, C.-W., and Gotelli, N.J. 2009. Sufficient sampling for asymptotic minimum species richness estimators. Ecology, **90**(4): 1125–1133. doi:10.1890/07-2147.1. PMID:19449706.

Chiarucci, A., Bacaro, G., Rocchini, D., and Fattorini, L. 2008. Discovering and rediscovering the sample-based rarefaction formula in the ecological literature. Community Ecol. **9**(1): 121–123. doi:10.1556/ComEc.9.2008.1.14.

Colwell, R.K. 2006. EstimateS software [online]. Available at http://viceroy.eeb.uconn.edu/EstimateS [accessed 29 July 2009].

Cuffney, T.F., Bilger, M.D., and Haigler, A.M. 2007. Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages. J. N. Am. Benthol. Soc. **26**(2): 286–307. doi:10.1899/0887-3593(2007)26[286:ATEOTC]2.0.CO;2.

Danz, N.P., Regal, R.R., Niemi, G.J., Brady, V.J., Hollenhorst, T.P., Johnson, L.B., Host, G.E., Hanowski, J.M., Johnston, C.A., Brown, T.N., Kingston, J.C., and Kelly, J.R. 2005. Environmentally stratified sampling design for the development of Great Lakes environmental indicators. Environ. Monit. Assess. **102**(1–3): 41–65. doi:10.1007/s10661-005-1594-8. PMID:15869177.

Darling, J.A., and Blum, M.J. 2007. DNA-based methods for monitoring invasive species: a review and prospectus. Biol. Invasions, **9**(7): 751–765. doi:10.1007/s10530-006-9079-4.

Fitzpatrick, M.C., Preisser, E.L., Ellison, A.M., and Elkinton, J.S. 2009. Observer bias and the detection of low density populations. Ecol. Appl. **19**(7): 1673–1679. doi:10.1890/09-0265.1. PMID:19831062.

Gotelli, N.J., and Colwell, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol. Lett. **4**(4): 379–391. doi:10.1046/j.1461-0248.2001.00230.x.

Grenouillet, G., Pont, D., and Seip, K.L. 2002. Abundance and species richness as a function of food resources and vegetation structure: juvenile fish assemblages in rivers. Ecography, **25**(6): 641–650. doi:10.1034/j.1600-0587.2002.250601.x.

Grigorovich, I.A., Colautti, R.I., Mills, E.L., Holeck, K., Ballert, A.G., and MacIsaac, H.J. 2003*a*. Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. Can. J. Fish. Aquat. Sci. **60**(6): 740–756. doi:10.1139/f03-053.

Grigorovich, I.A., Korniushin, A.V., Gray, D.K., Duggan, U.C., Colautti, R.I., and MacIsaac, H.J. 2003*b*. Lake Superior: an invasion cokdspot? Hydrobiologia, **499**(1–3): 191–210. doi:10.1023/A:1026335300403.

Grigorovich, I.A., Kelly, J.R., Darling, J.A., and West, C.W. 2008. The quagga mussel invades the Lake Superior basin. J. Great Lakes Res. **34**(2): 342–350. doi:10.3394/0380-1330(2008)34[342:TQMITL]2.0.CO;2.

Harvey, C.T., Qureshi, S.A., and MacIsaac, H.J. 2009. Detection of colonizing, aquatic, non-indigenous species. Divers. Distrib. **15**(3): 429–437. doi:10.1111/j.1472-4642.2008.00550.x.

Holeck, K.T., Mills, E.L., MacIsaac, H.J., Dochoda, M.R., Colautti, R.I., and Ricciardi, A. 2004. Bridging troubled waters: biological invasions, transoceanic shipping, and the Laurentian Great Lakes. Bioscience, **54**(10): 919–929. doi:10.1641/0006-3568(2004)054[0919:BTWBIT]2.0.CO;2.

Hulme, P.E. 2006. Beyond control: wider implications for the management of biological invasions. J. Appl. Ecol. **43**(5): 835–847. doi:10.1111/j.1365-2664.2006.01227.x.

Jerde, C.L., Mahon, A.R., Chadderton, W.L., and Lodge, D.M. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters, **4**(2): 150–157. doi:10.1111/j.1755-263X.2010.00158.x.

Kolar, C.S., and Lodge, D.M. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science (Washington, D.C.), **298**(5596): 1233–1236. doi:10.1126/science.1075753. PMID:12424378.

Lodge, D.M., Stein, R.A., Brown, K.M., Covich, A.P., Brönmark, C., Garvey, J.E., and Klosiewski, S.P. 1998. Predicting impact of freshwater exotic species on native biodiversity: challenges in spatial scaling. Aust. J. Ecol. **23**(1): 53–67. doi:10.1111/j.1442-9993.1998.tb00705.x.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle, P.B., Smith, M., Andow, D.A., Carlton, J.T., and McMichael, S. 2006. Biological invasions: recommendations for U.S. policy and management. Ecol. Appl. **16**(6): 2035–2054. doi:10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2. PMID:17205888.

Loo, S.E., Mac Nally, R., and Lake, P.S. 2007. Forecasting New Zealand mudsnail invasion range: model comparisons using native and invaded ranges. Ecol. Appl. **17**(1): 181–189. doi:10.1890/1051-0761(2007)017[0181:FNZMIR]2.0.CO;2. PMID:17479844.

MacKenzie, D.I., and Royle, J.A. 2005. Designing occupancy studies: general advice and allocating survey effort. J. Appl. Ecol. **42**(6): 1105–1114. doi:10.1111/j.1365-2664.2005.01098.x.

Magnuson, J.J., Benson, B.J., and McLain, A.S. 1994. Insights on species richness and turnover from long-term ecological research: fishers in north temperate lakes. Am. Zool. **34**: 437–451.

Mao, C.X., Colwell, R.K., and Chang, J. 2005. Estimating the species accumulation curve using mixtures. Biometrics, **61**(2): 433–441. doi:10.1111/j.1541-0420.2005.00316.x. PMID:16011689.

Mills, E.L., Leach, J.H., Carlton, J.T., and Secor, C.L. 1994. Exotic species and the integrity of the Great Lakes. Bioscience, **44**(10): 666–676. doi:10.2307/1312510.

Myers, J.H., Simberloff, D., Kuris, A.M., and Carey, J.R. 2000. Eradication revisited: dealing with exotic species. Trends Ecol. Evol. **15**(8): 316–320. doi:10.1016/S0169-5347(00)01914-5. PMID:10884695.

Palmer, M.W., McGlinn, D.J., and Fridley, J.D. 2008. Artifacts and artifictions in biodiversity research. Folia Geobot. **43**(3): 245–257. doi:10.1007/s12224-008-9012-y.

Peterson, G.S., Hoffman, J.C., Trebitz, A.S., West, C.W., and Kelly, J.R. 2011. Establishment patterns of non-native fishes: lessons from the Duluth–Superior harbor and lower St. Louis River, an invasion-prone Great Lakes coastal ecosystem. J. Great Lakes Res. **37**(2): 349–358. doi:10.1016/j.jglr.2011.03.009.

Rew, L.J., Maxwell, B.D., Dougher, F.L., and Aspinall, R. 2006. Searching for a needle in a haystack: evaluating survey methods for non-indigenous plant species. Biol. Invasions, **8**(3): 523–539. doi:10.1007/s10530-005-6420-2.

Ricciardi, A. 2001. Facilitative interactions among aquatic invaders: is an "invasional meltdown" occurring in the Great Lakes? Can. J. Fish. Aquat. Sci. **58**(12): 2513–2525. doi:10.1139/f01-178.

Ricciardi, A. 2006. Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. Divers. Distrib. **12**(4): 425–433. doi:10.1111/j.1366-9516.2006.00262.x.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D., and Hines, A.H. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. Am. Zool. **37**: 621–632.

Sheaves, M. 2009. Consequences of ecological connectivity: the coastal ecosystem mosaic. Mar. Ecol. Prog. Ser. **391**: 107–115. doi:10.3354/meps08121.

Smith, K.L., and Jones, M.L. 2005. Watershed-level sampling effort requirements for determining riverine fish species composition. Can. J. Fish. Aquat. Sci. **62**(7): 1580–1588. doi:10.1139/f05-098.

Smith, K.L., and Jones, M.L. 2008. Allocation of sampling effort to optimize efficiency of watershed-level ichthyofaunal inventories. Trans. Am. Fish. Soc. **137**(5): 1500–1506. doi:10.1577/T07-024.1.

Smokorowski, K.E., and Pratt, T.C. 2007. Effect of a change in physical structure and cover on fish and fish habitat in freshwater ecosystems — a review and meta-analysis. Environ. Rev. **15**: 15–41. doi:10.1139/a06-007.

St. Louis River Citizens Action Committee. 2002. Lower St. Louis River Habitat Plan. St. Louis River Citizens Action Committee, Duluth, Minnesota.

Stachowicz, J.J., Fried, H., Osman, R.W., and Whitlatch, R.B. 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology, **83**(9): 2575–2590. doi:10.1890/0012-9658(2002)083[2575:BIRAME]2.0.CO;2.

Steinberg, A.J., Sereres, C.S., Burrows, M., and MacIsaac, H.J. 2007. Temporal pattern of government funding for nonindigenous species research in the Great Lakes. J. Great Lakes Res. **33**(1): 136–142. doi:10.3394/0380-1330(2007)33[136:TPOGFF]2.0.CO;2.

Stevens, D.L., Jr., and Olsen, A.R. 1999. Spatially restricted surveys over time for aquatic resources. J. Agric. Biol. Environ. Stat. **4**(4): 415–428. doi:10.2307/1400499.

Stevens, D.L., Jr., and Olsen, A.R. 2004. Spatially-balanced sampling of natural resources in the presence of frame imperfections. J. Am. Stat. Assoc. **99**(465): 262–278. doi:10.1198/016214504000000250.

Stortz, K.R., and Sydor, M. 1980. Transports in the Duluth–Superior harbor. J. Great Lakes Res. **6**(3): 223–231. doi:10.1016/S0380-1330(80)72103-2.

Theobald, D.M., Stevens, D.L., White, D., Urquhart, N.S., Olsen, A.R., and Norman, J.B. 2007. Using GIS to generate spatially balanced random survey designs for natural resource applications. Environ.

Manage. **40**(1): 134–146. doi:10.1007/s00267-005-0199-x. PMID: 17546523.

Trebitz, A.S., Kelly, J.R., Hoffman, J.C., Peterson, G.S., and West, C.W. 2009. Exploiting habitat and gear patterns for efficient detection of rare and non-native benthos and fish in Great Lakes coastal ecosystems. Aquat. Invas. **4**(4): 651–667. doi:10.3391/ai.2009.4.4.10.

Trebitz, A.S., West, C.W., Hoffman, J.C., Kelly, J.R., Peterson, G.S., and Grigorovich, I.A. 2010. Status of non-indigenous benthic invertebrates in the Duluth–Superior Harbor and the role of sampling methods in their detection. J. Great Lakes Res. **36**(4): 747–756. doi:10.1016/j.jglr.2010.09.003.

Turner, A.M., and Trexler, J.C. 1997. Sampling aquatic invertebrates from marshes: evaluating the options. J. N. Am. Benthol. Soc. **16**(3): 694–709. doi:10.2307/1468154.

Vander Zanden, M.J., and Olden, J.D. 2008. A management framework for preventing the secondary spread of aquatic invasive species. Can. J. Fish. Aquat. Sci. **65**(7): 1512–1522. doi:10.1139/F08-099.

Vander Zanden, M.J., Hansen, G.J.A., Higgins, S.N., and Kornis, M.S. 2010. A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. J. Great Lakes Res. **36**(1): 199–205. doi:10.1016/j.jglr.2009.11.002.

Wolda, H. 1981. Similarity indices, sample size and diversity. Oecologia (Berl.), **50**(3): 296–302. doi:10.1007/BF00344966.

Appendix A

Table A1 follows.

Table A1. Variables used in the principal components analysis (PCA) and associated *k*-mean cluster analysis for the targeted area (SCS) design.

						Metadata	
Variable	Description	Units	Scale	Agency source	Program	Primary source	Additional source
1	Euclidean distance to nearest wetlands	Metres	1 : 24 000	US FWS; WI DNR	NWI; WWI	NWI wetland polygons	NWI wetland polygons
2	Euclidean distance to nearest road and railway within the study area	Metres	1 : 100 000	US EPA R5	Inland Sensitivity Atlas, Western Lake Superior	National rail network	US major roads
3	Euclidean distance to nearest disturbed plant community within the study area	Metres	1 : 24 000	Not apply	St. Louis River Alliance	Plant communities	
4	Euclidean distance to nearest coastal wetland community within the study area	Metres	1 : 24 000	Not apply	St. Louis River Alliance	Plant communities	
5	Cost distance to nearest PCS facility within the embayment	Metres		US EPA	Envirofacts database (NPDES)	NPDES	
6	Cost distance to nearest contaminated hotspot within the embayment	Metres	1 : 24 000	Not apply	St. Louis River Alliance	Contaminated sediments	
7	Cost distance to nearest harbor facility within the embayment	Metres	1 : 24 000	USE PA R5	St. Louis River Alliance; Inland Sensitivity Atlas, Western Lake Superior	Harbor facilities	Oil storage facilities
8	Cost distance to nearest dam within the embayment	Metres	1 : 24 000	Not apply	St. Louis River Alliance	Non-navigational dams	
9	Cost distance to nearest boat ramp within the embayment	Metres	1 : 24 000	Not apply	St. Louis River Alliance	Boat access	
10	Kernel density of harbor facilities	No. of facilities/km^2	1 : 24 000	US EPA R5	St. Louis River Alliance; Inland Sensitivity Atlas, Western Lake Superior	Harbor facilities	Oil storage facilities
11	Kernel density of PCS discharge facilities	No. of facilities/km^2		US EPA	Envirofacts database (NPDES)	NPDES	
12	Wetland area within a 250 m radius of sample	100 m^2	1 : 24 000	USFWS; WI DNR	NWI; WWI	NWI wetland polygons	NWI wetland polygons
13	Wetland area within a 2 km radius of sample	100 m^2	1 : 24 000	US FWS; WI DNR	NWI; WWI	NWI wetland polygons	NWI wetland polygons
14	Ratio of wetland area within a 2 km radius to euclidean distance to nearest wetland	100 m^2/m	1 : 24 000	US FWS; WI DNR	NWI; WWI	NWI wetland polygons	NWI wetland polygons
15	TIN bathymetry interpolated from 135665 NOS, US ACE, and NOS ENC sounding points and contours	Metres	1 : 24 000	NOAA; US ACE	NOAA and USACE hydrographic and navigation channel surveys	NOAA hydrographic surveys	Geoprocessing method
16	Fetch length with wind direction from 45° (NE)	Metres		NOAA; US ACE	Shoreline information	Calculated distance of open water from shoreline	Fetch calculation method
17	Fetch length with wind direction from 225° (SW)	Metres		NOAA; US ACE	Shoreline information	Calculated distance of open water from shoreline	Fetch calculation method
18	Fetch length with wind direction from 270° (W)	Metres		NOAA; US ACE	Shoreline information	Calculated distance of open water from shoreline	Fetch calculation method

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Table A1 (*concluded*).

Variable	Description	Units	Scale	Agency source	Program	Metadata	
						Primary source	Additional source
19	Variety of terrestrial plant communities within 250 m radius	Ordinal number	1 : 24 000	Not apply	St. Louis River Alliance	Plant communities	
20	Fine material in top sediment layer (%)	Percent	1 : 24 000	MPCA	MPCA Phase III GIS-based sediment quality database for the St. Louis River area of concern	MPCA sediment database	Sediment interpolation method
21	Total organic carbon concentration in top sediment layer (%)	Percent	1 : 24 000	MPCA	MPCA Phase III GIS-based sediment quality database for the St. Louis River area of concern	MPCA sediment database	Sediment interpolation method

Note: All data were derived from publicly available geographic information system (GIS) information. Distance metrics were calculated using standard GIS techniques and modeling. We measured distance to nearest wetlands, as well as pollution sources based on the U.S. Environmental Protection Agency (EPA) permit compliance system (PCS). The first 11 variables were considered to relate to potential non-native species introduction vectors and the second 10 were considered to relate to habitat attributes. Agency sources include the U.S. Fish and Wildlife Service (US FWS), the Wisconsin Department of Natural Resources (WI DNR), the U.S. Environmental Protection Agency Region 5 (US EPA R5), the National Oceanic and Atmospheric Administration (NOAA), the U.S. Army Corps of Engineers (US ACE), and the Minnesota Pollution Control Agency (MPCA). Data were taken from multiple programs, including the National Pollutant Discharge Elimination System (NPDES), the National Wetlands Inventory (NWI), and the Wisconsin Wetlands Inventory (WWI). Bathymetry data were obtained from the US ACE and the National Ocean Service electronic navigational chart (NOS ENC).

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Seasonal shift in the effects of predators on juvenile Atlantic salmon (*Salmo salar*) energetics

Darren M. Ward, Keith H. Nislow, and Carol L. Folt

Abstract: Predator effects on prey populations are determined by the number of prey consumed and effects on the traits of surviving prey. Yet the effects of predators on prey traits are rarely evaluated in field studies. We measured the effects of predators on energetic traits (consumption and growth rates) of juvenile Atlantic salmon (*Salmo salar*) in a large-scale field study. Salmon fry were released at 18 sites that encompassed a wide range of predatory slimy sculpin (*Cottus cognatus*) abundance. We sampled salmon after 21 and 140 days to measure salmon growth and estimate consumption using a mass-balance model of methylmercury accumulation. Salmon population density was reduced fivefold at sites with abundant sculpin. Over the early season, salmon consumed less where sculpin were abundant, suggesting that reduced foraging under predation risk contributed to predator-caused mortality. In contrast, over the late season, salmon grew more where sculpin were abundant, suggesting that compensatory growth at reduced salmon population density moderated predator-caused mortality. Predator effects on prey energetics can drive variation in survival and growth, with important consequences for population dynamics.

Résumé : Les effets des prédateurs sur les populations de proies sont déterminés par le nombre de proies consommées et les effets sur les traits des proies survivantes. On évalue, néanmoins, rarement les effets des prédateurs sur les traits des proies dans les études sur le terrain. Nous mesurons les effets des prédateurs sur les traits énergétiques (taux de consommation et de croissance) de jeunes saumons atlantiques (*Salmo salar*) dans une étude de terrain à grande échelle. Nous avons libéré des alevins de saumons dans 18 sites qui couvrent une grande étendue d'abondances de prédateurs, des chabots visqueux (*Cottus cognatus*). Nous avons échantillonné les saumons après 21 et 140 jours afin de mesurer la croissance des saumons et d'estimer leur consommation à l'aide d'un modèle de bilan massique d'accumulation de méthylmercure. La densité des saumons a été réduite par un facteur de cinq aux sites où les chabots sont abondants. Durant le début de la saison, les saumons consomment moins là où les chabots sont nombreux, ce qui laisse croire que la recherche réduite de nourriture à cause du risque de prédation contribue à la mortalité due aux prédateurs. En revanche, durant la fin de la saison, les saumons croissent plus lorsque les chabots sont abondants, ce qui indique qu'une croissance compensatoire aux densités réduites de saumons tempère la mortalité due aux prédateurs. Les effets des prédateurs sur l'énergétique des proies peuvent expliquer les variations de la survie et de la croissance, qui ont des conséquences importantes sur la dynamique de la population.

[Traduit par la Rédaction]

Introduction

Heavy predation on small, juvenile size classes directly reduces recruitment of fish cohorts (Hixon and Beets 1993; Walters and Juanes 1993). In addition to direct losses to predators, predation can have a strong effect on average behavioral and physiological traits of prey cohorts. For example, predators can alter the average energetic traits (consumption and growth rates, activity costs) of a cohort through at least three distinct processes (Relyea 2002): first, behavioral responses of prey to predation risk can result in reduced time spent foraging or elevated energetic costs; second, predator-caused mortality may be biased toward a specific subset of the prey population, such as smaller individuals or more active foragers; third, there may be reduced competition among prey after predators reduce prey population density. These effects of predators on prey traits can have important consequences for population dynamics of prey (Lima 1998; Peckarsky et al. 2008), particularly for species like salmonids (trout and salmon), where survival, fecundity, and the pattern of life history expression are strongly tied to individual consumption and growth rates

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(Metcalfe 1998). Yet the strength and mechanisms of predator effects on prey traits are rarely evaluated in field studies of predator effects on prey.

Atlantic salmon (*Salmo salar*) fry are very vulnerable to predators during a critical period early in their first growing season, when they disperse from natal habitat and transition from dependence on yolk reserves to exogenous feeding (Henderson and Letcher 2003; Ward et al. 2008*a*). Survival during this critical period can largely determine total cohort recruitment for Atlantic salmon (Milner et al. 2003; Nislow et al. 2004) and other stream salmonids (Elliott 1989; Lobón-Cerviá and Rincón 2004). While direct losses to predators can be high, short-term (minutes to hours) behavioral studies show that foraging and activity of juvenile Atlantic salmon are also strongly affected by predation risk (Gotceitas and Godin 1991). Under predation risk, salmon reduce foraging and spend more time hiding in the substrate (Leduc et al. 2004). Yet because of the challenges of estimating longer-term (days to weeks) consumption rates in the field, it is not clear whether the short-term behavioral response to predators is associated with reduced foraging over the critical period. Small-scale studies in field enclosures (Blanchet et al. 2008) and the laboratory (Orpwood et al. 2006; Blanchet et al. 2007) suggest that behavioral compensation may allow salmon to maintain long-term consumption and growth rates despite short-term predator avoidance behavior. Testing the link between predation risk and critical period consumption in the field over larger spatial and temporal scales is crucial because previous work suggests that suppressed consumption over the critical period is a key indicator of sites with poor early survival for Atlantic salmon (Kennedy et al. 2004, 2008).

While the peak vulnerability of newly emerging or newly stocked Atlantic salmon fry to fish predators only lasts a few days (Henderson and Letcher 2003; Ward et al. 2008*a*), the effects of predators on salmon traits may persist through the growing season (Ward and Hvidsten 2010). High early predation losses could suppress mean growth over the season if early predation mortality is biased towards inherently active foragers. Alternatively, high early predation losses could lead to higher mean growth over the growing season owing to reduced competition at lower salmon population density. Increased mean growth as a response to early predation loss could be a powerful demographic compensation mechanism for Atlantic salmon populations (Vincenzi et al. 2008; Horton et al. 2009) and is consistent with numerous recent observations of density-dependent growth of juvenile Atlantic salmon (Imre et al. 2005; Ward et al. 2009), yet remains untested in the field.

The goal of this study was to evaluate the relationship between predator abundance and survival, growth, and consumption rates of juvenile Atlantic salmon in the field. We were particularly interested in assessing the energetic effects of predators and predation risk over longer time scales than are typically considered in behavioral studies of prey responses to predation risk. This has long been technically challenging for field studies owing to the practical difficulty in measuring time-integrated consumption rates of individual fish over days and weeks. We addressed this challenge by estimating prey consumption using a mass-balance model of methylmercury accumulation, an approach that yields robust, time-integrated individual consumption estimates over periods of weeks to months (Trudel et al. 2000; Ward et al. 2010*a*). This approach also allowed us to separate the effects of predators during the early critical period (~20 days after stocking) from long-term or delayed effects over the rest of the growing season (~140 days after stocking) by sampling fish for growth and consumption estimates at these two separate time intervals.

Materials and methods

We conducted the field study at 18 study sites located on six small (<7 m mean summer width) tributary streams of the Connecticut River in Grafton and Sullivan counties in New Hampshire, USA (three sites per stream; general habitat descriptions in Ward et al. 2008*b*, 2009). All sites on the same tributary were separated by at least 1 km; all tributaries were separated by at least 3 km along the main stem of the Connecticut River or Mascoma River (a larger Connecticut River tributary). The study streams had predominantly forested watersheds and mostly gravel and cobble substrate. Fish communities in the study streams consisted of stocked Atlantic salmon (including an overyearling juvenile cohort from previous years of stocking), brook trout (*Salvelinus fontinalis*), slimy sculpin (*Cottus cognatus*), and minnows (primarily *Rhinichthys atratulus* and *Rhinichthys cataractae*). Our previous work in these streams shows that, of these species, only slimy sculpin are an important determinant of first-summer survival for Atlantic salmon (Ward et al. 2008*a*, 2008*b*). Stomach sampling of slimy sculpin revealed that the negative effect of slimy sculpin on Atlantic salmon survival was due at least in part to sculpin predation on salmon fry in the first few days after stocking (Ward et al. 2008*a*).

On 8–9 May 2007, we released 2000 Atlantic salmon fry at each study site. Fish stocking was conducted as part of an ongoing restoration program for Atlantic salmon in New England (Folt et al. 1998). The salmon were produced at the White River National Fish Hatchery in Bethel, Vermont, and were stocked as unfed fry that had not yet transitioned from yolk resources to independent feeding. We released the fry in 10–15 cm deep water over gravel and small cobble substrate. Fry were released into a stilling well (plastic bucket with the bottom removed) placed over the substrate to protect them from being washed downstream before finding shelter in the substrate. All fry were released at a single location at each study site. We collected a subsample of fry at the time of stocking to measure initial size (0.16 ± 0.03 g; mean ± SD) and mercury concentration (24 ± 2 ppb dry).

There is no natural Atlantic salmon reproduction in the study streams, as dams on the main stem of the Connecticut River prevent adult return. Further, study sites within a stream were far enough apart that movement of stocked salmon among sites during their first summer was not likely (Ward et al. 2008*b*). Therefore, we assumed that the young-of-the-year salmon we sampled throughout the summer growing season at each site were from our controlled stocking events.

Fish sampling was conducted in three bouts over the growing season by electrofishing (Smith-Root BP-12 electrofisher at 300–500 V DC) and was conducted under Dartmouth College Animal Care and Use Program protocol 06-02-12. We collected fish from each site for growth and mercury analysis

on two dates, timing sampling to capture conditions just after the critical period (29–30 May 2007, ~20 days after stocking) and at the end of the summer growing season (25–26 September 2007, ~140 days after stocking). These fish were collected in a single electrofishing pass ~20–50 m long at the stocking site. We could not conduct a full population density estimate at all sites during these samples and still complete the time-critical growth and consumption sampling within a short time window, so we conducted a separate sample bout to measure the population density of Atlantic salmon, slimy sculpin, and the rest of the fish community at each site from 3 to 30 August 2007. For population density estimates, we fished three 30 m sample reaches at each study site, with 30 m between reaches (150 m total stream length in the sampled area). Each 30 m reach was isolated with block nets at the upstream and downstream end and fished for two to four passes of removal sampling. All salmon and a subset of all other species were measured to the nearest millimetre (total length). We used a maximum weighted likelihood technique to estimate total abundance of each species in each plot from removal data (Carle and Strub 1978). We separated young-of-the-year salmon from overyearlings based on stream-specific length distributions and estimated density separately for these age classes. Density estimates for slimy sculpin include only individuals >55 mm, based on the minimum-sized sculpin that we have found containing salmon fry in stomach sampling. Atlantic salmon population density in the sample plots is a function of survival of stocked fish and emigration from the study area, but spatially extensive sampling upstream and downstream of sites stocked by the same techniques in previous years showed that population density within 100 m of the stocking site is a reliable index of total first-summer survival of point-stocked Atlantic salmon fry (Ward et al. 2008*b*).

Mayfly (order Ephemeroptera, family predominantly Baetidae) nymphs were collected for mercury analysis to assess mercury concentrations in salmon prey. Mayflies were by far the dominant prey in diets of Atlantic salmon collected in early-season samples (mean percentage of diet by numbers: 74% ± 19% (mean ± SD)) and remained a substantial component of the diet through the late-season samples (28% ± 22%). Further, mayfly mercury concentrations are representative of other abundant aquatic insects at our study sites (D.M. Ward, unpublished data). At each site, we collected three replicate mayfly samples using an electrobugging technique (Taylor et al. 2001). Each sample consisted of three discrete subplots (~1 m × 0.3 m) treated with a 10 s sweep with the electrofisher anode (300–500 V DC); stunned insects drifted into a 500 µm mesh Surber net held downstream. This technique yielded sufficient biomass of mayflies (2–17 mg dry mass) for mercury analysis with little detritus. We also used the mean total biomass of mayflies captured via standardized electrobugging as an index of prey biomass available at the study sites.

All biological samples collected for mercury analysis were stored on ice in acid-cleaned vials or sample bags for transportation. We weighed and measured fish and removed their stomach contents before freezing them for storage. Stomach contents were preserved in 70% ethanol and sorted to family level to estimate diet composition. Mayflies were sorted from invertebrate samples within 24 h and frozen for storage. Frozen fish and mayfly samples were freeze-dried, and a 0.1 g homogenized subsample (fish) or the entire sample mass (mayflies) was digested for mercury analysis. Following our established protocols (Chen et al. 2000; Ward et al. 2010*b*), all samples were digested in ultraclean nitric acid in sealed, acid-cleaned Teflon vessels in a microwave reaction accelerator. Total mercury concentrations in the digested solution were measured by inductively coupled plasma mass spectrometry. Quality control was ensured by analysis of certified reference materials (NIST 2976, mussel tissue; CRC DORM-2, dogfish muscle), duplicate samples, and digestion blanks with every processing batch of 20 samples.

We measured total mercury concentrations in all samples, but methylmercury is the mercury compound that is most prevalent in fish and most prone to bioaccumulation. Variation in the proportion of total mercury that is methylmercury could alter mercury accumulation dynamics and the model parameters required to estimate consumption from the mass-balance model (Lepak et al. 2009). To assess potential variation in methylmercury, we measured a subset of samples for mercury speciation (one fish and one prey sample from each site). Mercury speciation samples were measured by isotope dilution gas chromatography – inductively coupled plasma mass spectrometry (Taylor et al. 2008). In all fish samples, nearly all the mercury was methylmercury (97% ± 2% (mean ± SD); range 94%–100%), as observed in numerous other studies (Bloom 1992). In prey samples, the mean proportion of mercury as methylmercury was lower, but there was no consistent variation across streams (86% ± 6% (mean ± SD); range 76%–94%). We assumed that the mean proportions were consistent across all sites, to use literature parameters for methylmercury accumulation in our mercury accumulation model.

Mercury mass-balance model

We used a simple, widely used contaminant accumulation model (Forseth et al. 1992; Rowan and Rasmussen 1996) to estimate consumption rates for young-of-the-year Atlantic salmon. The model, as adapted for stable isotopes and non-reproductive fish, is described in detail elsewhere (Trudel et al. 2000; Kennedy et al. 2004). Briefly, as fish accumulate methylmercury largely from the prey they consume, total prey consumption (C, g·g^{-1}·day^{-1}) can be estimated from initial methylmercury body burden (B_1, ng), the final methylmercury body burden (B_{1+d}, where d is the number of days in the interval), the methylmercury concentrations in prey (F, ng·g^{-1}), and literature estimates for assimilation efficiency (a, proportion) and elimination rate (E, proportion).

For chronically exposed fish, methylmercury elimination rate depends on both temperature and body size as $\ln(E) = 0.066T - 0.2\ln(W) - 5.83$, where T is temperature (°C) and W is mass (g) (Trudel and Rasmussen 1997). As temperature and body size varied over time, we iterated the model on a daily time step with mean daily temperature for each site from field data (hourly measurements by temperature loggers anchored to the stream bed; Onset Optic StowAway, Onset Computer Corporation, Pocasset, Mass., USA) and individual daily size estimated by assuming constant instantaneous growth rate between sampling periods. Growth was calculated as $\ln(W_{1+d}W_1^{-1})\cdot d^{-1}$, where W_{1+d} is individual mass at sampling, and W_1 is mean mass at stocking (for the early sea-

son) or site-mean mass in the early sample (for the late season). Thus, the daily model for mercury body burden was $B_{t+1} = B_t + aCWF - EB_t$, with B_{t+1} carried over as B_t for the subsequent day. Measured total mercury concentrations were converted to methylmercury as described above. We used an iterative procedure (Hood 2008) to identify the mean daily consumption rate that produced the observed final mercury body burden for each individual after the appropriate number of days (21 days for stocking to early season, 119 days for early season to late season).

For the early-season model and growth calculations, we used the mean size and mercury concentration of fry sampled prior to stocking as initial conditions. All fry originated from the same source with uniform initial mean size and mercury concentration across sites, so this approach does not affect site comparisons. For the late-season model and growth calculations, we used the mean size and mercury concentration of fish at the early sampling date as initial conditions. This approach assumes that the variation in size and mercury concentration across sites is very large compared with the variation among individuals within sites, otherwise site mean values would not be appropriate as initial conditions for individuals in the late-season sample. In our early-season sample, 73% of the variation in size and 95% of the variation in mercury concentration was across sites, suggesting that this was a reasonable approximation.

Data analysis

Our analysis assumes that large differences among sites in survival during the early critical period generate variation in salmon population density that persists through the growing season, as seen for other Atlantic salmon populations (Milner et al. 2003; Nislow et al. 2004) and other stream salmonids (Elliott 1989; Lobón-Cerviá and Rincón 2004). Consistent with this assumption, catch per effort of young-of-the-year salmon during the early-season sample collection was significantly correlated with density from the intensive population samples later in the season, indicating that the density gradient across sites was established early in the season (r = 0.73, n = 18, P = 0.0005). Therefore, we treat the mean population density of the three sample plots fished at each site in August as an index of early survival during the critical period (for survival analyses) as well as a measure of the population density salmon experience between the early- and late-season samples (for density–growth analyses).

Our primary analysis focused on the relationship between predator abundance and mean energetic traits of Atlantic salmon. We first used linear regression to test whether salmon population density was suppressed at high sculpin density. For both the early- and late-season data sets, we used linear regression to determine whether mean salmon prey consumption and growth rates were suppressed at high sculpin density, as predicted for reduced foraging under predation risk or trait-biased predation, or whether consumption and growth were elevated at high sculpin density, as predicted for a compensatory response of salmon to reduced competition. We also used regression to test the direct relationship between salmon population density and late-season growth and consumption. For most regressions, fish population densities were $\log_{10}$-transformed to equalize variance and linearize relationships ($\log_{10}(x+1)$ for sculpin, which were absent from some sites).

For the primary analysis, our focus was on evaluating specific links between energetic traits and predator abundance, but prey consumption and growth rates can be affected by a suite of factors that affect energetic demand, including temperature, water chemistry, prey availability, and physical habitat. To assess the effects of these factors, we conducted an additional multimodel analysis for early- and late-season consumption and growth rates. For each response, we fit all possible regression models with slimy sculpin population density ($\log_{10}$-transformed) along with stream gradient (as a percentage, mean of the 30 m sample plots at each site), water temperature (in °C, seasonal mean of hourly measures from in-stream probes installed at each site), depth (in cm, mean of nine transects at each site), mean pH (seasonal mean of biweekly samples), and prey biomass ($\log_{10}$ mean mg dry mass of mayflies in electrobugging samples) as potential predictors. Consumption rate was also included as a predictor for growth rate. We ranked models according to small sample size corrected Akaike information criterion (AIC_c) and report the model-averaged parameter estimates and post hoc probability for each predictor with an AIC_c weight cutoff of 0.95 (Burnham and Anderson 2004).

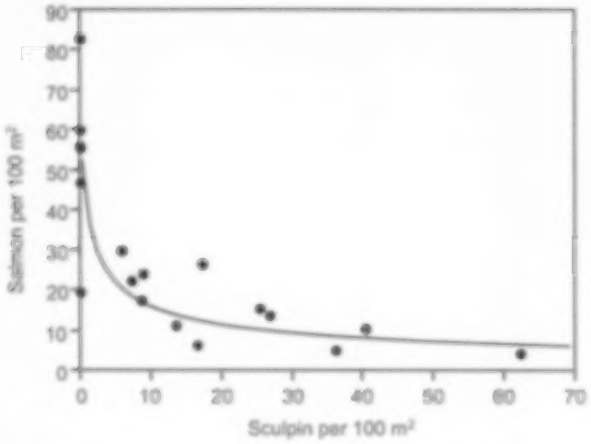
Results

The wide range in sculpin population density (0–60 sculpin per 100 m^2) and other conditions across the 18 study locations produced a correspondingly wide range in population density of juvenile Atlantic salmon (range in August: 4–83 salmon per 100 m^2). Mean performance as indicated by mass-balance estimated consumption and mean individual growth rates also varied widely across sites. Early-season consumption estimates ranged from 0.10 to 0.37 g·g^{-1}·day^{-1} and growth from 0.02 to 0.08 g·g^{-1}·day^{-1}, producing mean sizes in May ranging from 0.4 to 1.1 g across sites. Late-season consumption estimates ranged from 0.08 to 0.19 g·g^{-1}·day^{-1} and growth from 0.01 to 0.02 g·g^{-1}·day^{-1}, producing mean sizes in September ranging from 3.1 to 7.6 g across sites.

Population density of salmon fry was much lower at sites with abundant sculpin (Fig. 1; $\log_{10}$(salmon per 100 m^2) = 1.72 – 0.50($\log_{10}$(sculpin per 100 m^2 + 1)), R^2 = 0.73, root mean square error (RMSE) = 0.21, $F_{[1,16]}$ = 43.3, P < 0.0001). Consistent with reduced foraging under sculpin predation risk, salmon early-season prey consumption was also suppressed at sites with abundant sculpin (Fig. 2*a*; consumption (g·g^{-1}·day^{-1}) = 0.25–0.06($\log_{10}$(sculpin per 100 m^2 + 1)), R^2 = 0.38, RMSE = 0.06, $F_{[1,16]}$ = 9.7, P = 0.007). However, mean early-season growth of salmon was not related to sculpin abundance (Fig. 2*b*; R^2 = 0.01, RMSE = 0.02, $F_{[1,16]}$ = 0.002, P = 0.97).

Consistent with a compensatory response of salmon to reduced competition, late-season mean individual performance was elevated at sites with abundant sculpin and low salmon population density. Mean late-season growth was fastest at sites with low salmon population density (Fig. 3; growth (g·g^{-1}·day^{-1}) = 0.022–0.004[$\log_{10}$(salmon per 100 m^2)], R^2 = 0.43, RMSE = 0.002, $F_{[1,16]}$ = 11.9, P = 0.003), resulting in a positive relationship between late-season salmon growth and sculpin density (Fig. 2*d*; growth (g·g^{-1}·day^{-1}) = 0.016+

Fig. 1. Atlantic salmon summer population density from standardized stocking related to slimy sculpin population density. Each point is the mean population density from three 30 m sample reaches at each study site. The line is the regression fit to $\log_{10}$-transformed data.



0.00008(sculpin per 100 m^2), $R^2 = 0.36$, RMSE = 0.002, $F_{[1,16]} = 8.9$, $P = 0.009$). However, mean late-season prey consumption was not significantly elevated at sites with abundant sculpin (Fig. 2*c*; $R^2 = 0.09$, RMSE = 0.027, $F_{[1,16]} = 1.6$, $P = 0.23$). Thus, salmon experiencing low population density grew faster than salmon at high population density without consuming more prey, suggesting that energetic costs were reduced at low population density. This pattern in late-season growth drove variation in final size of salmon across sites, such that salmon were larger at the end of summer at sites with abundant sculpin ($\log_{10}$ mean mass (g) = 0.67 + 0.004(sculpin per 100 m^2), $R^2 = 0.42$, RMSE = 0.08, $F_{[1,16]} = 11.5$, $P = 0.003$) and low salmon population density ($\log_{10}$ mean mass (g) = 0.89–0.17[$\log_{10}$(salmon per 100 m^2)], $R^2 = 0.44$, RMSE = 0.08, $F_{[1,16]} = 12.6$, $P = 0.003$).

Beyond the effects of predators and population density, salmon consumption rates were most strongly correlated with mean prey biomass, with higher consumption rates at sites with higher prey biomass in both the early and late season (Table 1). Stream depth and gradient may also explain some variation in early-season consumption, with consumption greatest at deeper and lower-gradient sites, potentially reflecting drivers of profitable foraging habitat. Increased prey consumption was associated with faster growth in the early season, but not in the late season, suggesting that variation in late-season growth was associated with energetic expenditures, not intake (Table 1). Other factors that explained variation in growth rate include pH, with suppressed growth at acidic sites in early season and the reverse in the late season, suggesting a potential compensatory effect. The effect of sculpin population density on late-season growth reflects the closely correlated effect of salmon population density discussed above.

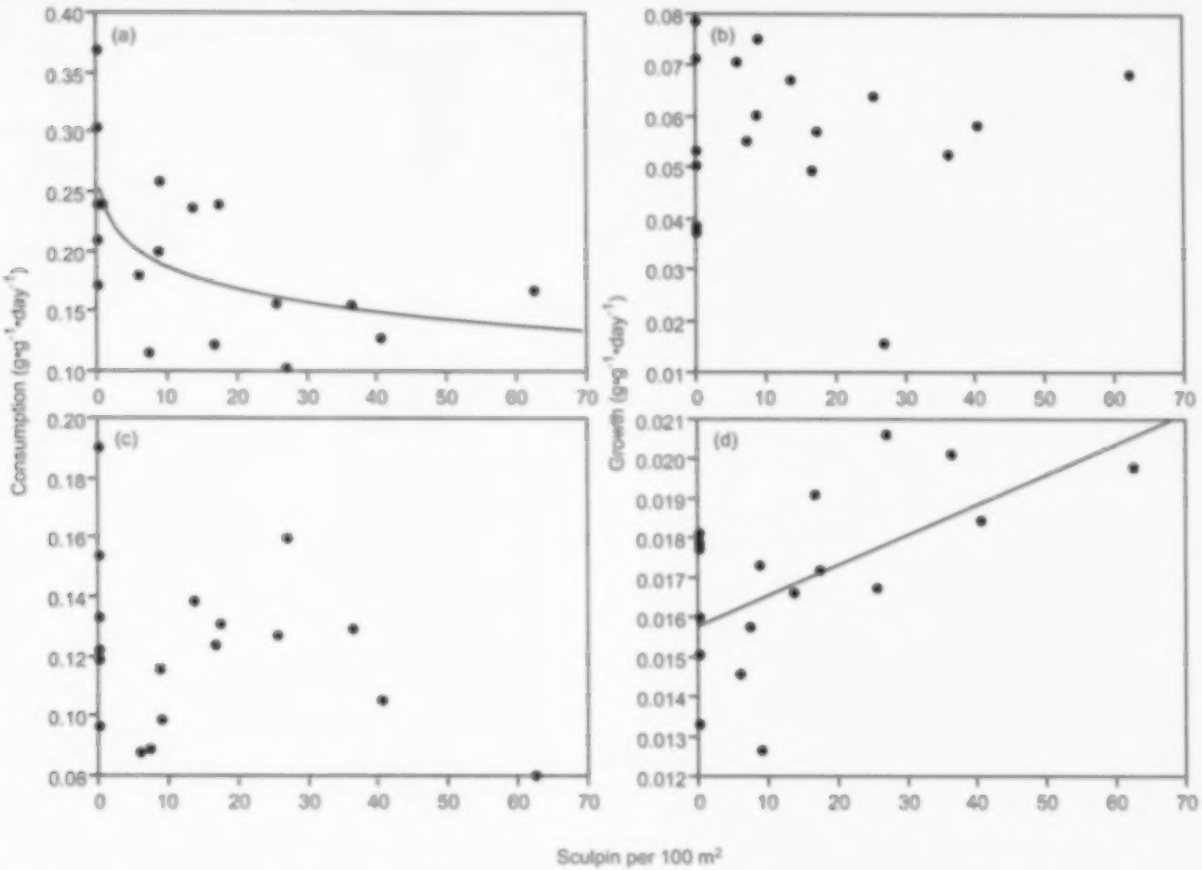
Discussion

Using robust, trace-element derived consumption rates of juvenile Atlantic salmon in the field, combined with controlled stocking across spatial and temporal variation in the biotic and abiotic environment, we were able to elucidate some of the basic mechanisms underlying variation in growth and survival of juvenile stream salmonids. In combination with our earlier work documenting sculpin predation on salmon fry (Ward et al. 2008*a*, 2008*b*), these results suggest that slimy sculpin predation strongly increased salmon mortality, likely compounded by a short-term effect on salmon traits (early-season consumption) but moderated by a delayed compensatory effect on salmon traits (late-season growth). These results are consistent with the idea that predator effects on prey traits are ubiquitous (Lima 1998) and that these effects are important for determining the effects of predators on populations (Werner and Peacor 2003; Peckarsky et al. 2008). Further, our seasonal sampling provides a compelling example of a temporal reversal of such effects.

Use of contaminant accumulation models to estimate consumption rates of free-living fish in the field is becoming increasingly common (Trudel et al. 2001; Rennie et al. 2008). These models have been shown to yield consumption estimates similar to those obtained with techniques that rely on direct measurement of stomach contents (Trudel et al. 2000) and capture ecologically meaningful differences across locations (Kennedy et al. 2004, 2008). A primary advantage of the contaminant mass-balance approach over standard bioenergetics models is the estimation of integrated consumption rates that are not confounded by problematic assumptions about the proportion of total energy consumed that is allocated to growth versus activity (Chipps and Wahl 2008) and so allow for assessment of the relative importance of energy intake and energy expenditure in driving patterns of growth (Rennie et al. 2005). For example, the factors that we identified in the multimodel analysis as important drivers of growth variation after accounting for variation in consumption may act by mediating energetic costs of maintaining osmotic balance (e.g., effect of pH in the early season) or activity costs associated with behavior or habitat use (e.g., effect of sculpin population density in the late season, reflecting density-dependent growth).

The utility of the contaminant mass-balance model approach for answering ecological questions about energy acquisition and use is not without limits. Accurate estimates of consumption require reliable estimates of model parameters. Of particular importance is adequate representation of contaminant concentration in the diet (Kennedy et al. 2004). Site-level bias in the estimate of contaminant concentrations in prey will confound site-level estimates of mean consumption rate of fish. Here, we used mercury in mayflies as a surrogate for the salmon diet, as they were a dominant prey item, they reflect mercury concentrations in other aquatic insect prey for juvenile salmon (Ward et al. 2010*a*), and contaminant accumulation in salmon generally tracks that in mayflies across sites (Ward et al. 2010*b*, 2010*c*). This approach is only valid as long as salmon did not disproportionately consume prey with very distinct mercury concentrations from mayflies at some sites. However, we found no evidence of a shift in the use of mayflies along the sculpin population gradient that could explain the pattern of reduced consumption under predation risk that we observed (correlation of proportion of mayflies in the diet with sculpin population

Fig. 2. Early-season (*a*, *b*) and late-season (*c*, *d*) consumption (*a*, *c*) and growth rates (*b*, *d*) of juvenile Atlantic salmon related to slimy sculpin population density. Each consumption or growth estimate is the mean of four or five salmon sampled at each study site; sculpin population density is the mean from three 30 m sample reaches at each study site. Regression lines are shown for significant fits to $\log_{10}$-transformed (*a*) or untransformed (*d*) data.

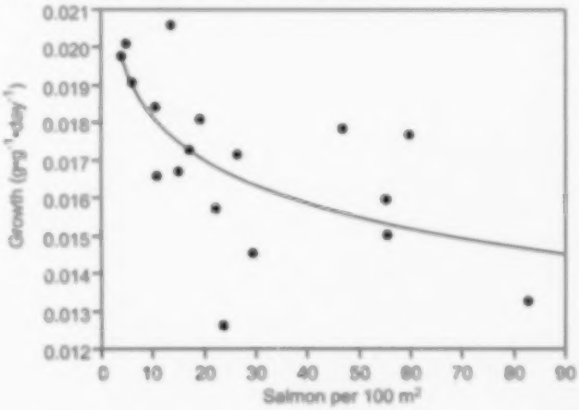


density; early season: $r = 0.25$, $P = 0.31$; late season: $r = 0.05$, $P = 0.84$).

Early season

Our results are particularly relevant to understanding the functional role of predators during early life history of stream-dwelling salmonids. This role has been difficult to elucidate. On one hand, small postemergent fry appear to be highly vulnerable to predatory fish (Ward et al. 2008*a*, 2008*b*), which are the likely major predators of juveniles before they become large enough to be preyed on by birds or mammals (Ward and Hvidsten 2010). At the same time, peak vulnerability may only extend for a brief period after emergence (Brännäs 1995; Henderson and Letcher 2003), potentially limiting the total predation rate. We have observed in this and previous studies that locations containing predatory sculpins have lower salmon first-summer recruitment rates than low-sculpin or sculpin-free sites (Ward et al. 2008*a*, 2008*b*). Yet when initial densities of salmon fry are very high, observed sculpin predation rates do not seem able to explain the magnitude of this decrease in survival (Ward et al. 2008*b*). This potential mismatch could be explained if

Fig. 3. Late-season growth rates of juvenile Atlantic salmon related to salmon population density. Each growth estimate is the mean of five salmon sampled at each study site, salmon population density is the mean from three 30 m sample reaches at each study site. The line is the regression fit to $\log_{10}$-transformed data.



predators had other negative effects in addition to direct predation, such as the reduced consumption rates that we observed.

Effects of predators on consumption rate during the critical period are likely to be particularly important for young-of-the-year Atlantic salmon. Previous research on Atlantic salmon survival through the critical period indicates that increased foraging opportunity (Nislow et al. 1998, 1999) or consumption rate (Kennedy et al. 2004, 2008) is associated with increased survival. Somewhat surprisingly, despite suppressed consumption at high predator density, we did not observe direct effects of predators on growth of juvenile salmon in the early season. However, increased early-season consumption was associated with rapid growth after accounting for variation in abiotic factors, particularly pH. Further, the absence of a simple negative relationship between predation risk and growth is consistent with the observation in previous studies that critical-period stressors are manifest by effects on survival and not individual growth (Einum et al. 2006; Kennedy et al. 2008). Reduced activity costs for fish that seek shelter to avoid predators might to some extent offset effects of reduced consumption on growth (Orpwood et al. 2006; Blanchet et al. 2008). Finally, while we cannot determine whether the effects of sculpin on mean consumption rates that we observed are the result of reductions in foraging rate or selective predation on individuals with higher foraging rates, the net result is the same — reductions in overall survival during an early critical period.

Late season

Low salmon population density in streams with abundant sculpin was associated with high growth rates during the late season, yielding mean salmon mass up to 50% larger at the end of summer in streams with abundant sculpin than in those without sculpin. This finding is concordant with a large body of work demonstrating strong density dependence of stream salmonid growth rates (Grant and Imre 2005; Ward et al. 2009). However, this is the first study to explicitly link predator-associated early mortality of salmon to increased later growth of survivors. This late-season, density-dependent growth has potentially important implications for the effects of predators on salmon populations. Fast-growing salmon migrate to sea sooner, and males mature earlier than slow growers (Letcher and Terrick 1998; Horton et al. 2009), suggesting the potential for strong compensation for early predator losses via increased individual growth (Ward and Hvidsten 2010).

While density-dependent growth of stream-dwelling salmonids clearly occurs, the precise mechanisms remain controversial (Ward et al. 2007). Our measured consumption rates indicate that energetic costs play an important role in determining the effect of changes in population density on growth. Consumption rates were not elevated at low-density, high-growth sites, suggesting that reduced energetic costs were the likely mechanism underlying increased individual growth at low density. While we cannot eliminate the possibility that shifts in diet composition or other factors drive the growth effect, reduced energetic costs are consistent with recent laboratory studies showing that juvenile salmonids respond to increases in resources or reduction in population density by reducing energetic expenditures rather than increasing their

Table 1. Model-averaged regression coefficients, standard errors (SE), and the post hoc probability that the coefficient does not equal zero for predictors of seasonal growth and consumption by Atlantic salmon.

	Early consumption		Late consumption		Early growth		Late growth	
	Coefficient (SE)	$P \neq 0$	Coefficient (SE)	$P \neq 0$	Coefficient (SE)	$P \neq 0$	Coefficient (SE)	$P \neq 0$
Prey biomass	**0.2 (0.06)**	1	**0.07 (0.03)**	1	0.006 (0.006)	0.6	−0.002 (0.002)	0.6
Stream gradient	*−0.008 (0.007)*	0.7	0.0004 (0.003)	0.1	−0.0004 (0.0009)	0.3	0.00002 (0.0002)	0.1
Mean depth	**0.009 (0.003)**	1	−0.00003 (0.0005)	0	0.00008 (0.0002)	0.3	0.000003 (0.00004)	0.1
Mean temperature	−0.00005 (0.006)	0	*−0.004 (0.005)*	0.5	0.0005 (0.001)	0.3	0.00004 (0.0003)	0.1
Sculpin density	**−0.05 (0.02)**	1	−0.0004 (0.003)	0.1	*0.001 (0.002)*	0.5	**0.001 (0.0006)**	0.9
pH	0.005 (0.01)	0.2	**−0.02 (0.02)**	0.8	**0.05 (0.008)**	1	**−0.002 (0.001)**	0.9
Early consumption	—	—	—	—	**0.04 (0.02)**	0.9	—	—
Late consumption	—	—	—	—	—	—	0.009 (0.01)	0.6

Note: Missing coefficients indicate that the predictor was not included in the model. Coefficients in bold are for predictors included in the best model for each response (lowest corrected Akaike information criterion (AIC$_c$) values: −52.8 for early consumption, −79.1 for late consumption, −115.1 for early growth, −168.2 for late growth); coefficients in italics are for predictors included in additional models within 2 AIC$_c$ units of the best model (ΔAIC$_c$ values: 1.3 for early consumption, 1.5 for late consumption, 1.0 for early growth, 2.1 for late growth).

prey intake rates (Orpwood et al. 2006; Kaspersson et al. 2010). Interestingly, our field studies (Ward et al. 2009; current study) suggest that, in contrast to reduced competitor density, increased prey availability is associated with increased growth and prey consumption. This suggests that fundamentally different mechanisms underlie growth responses to increased resource availability versus increased competition for a given level of resources: the relative scope for compensatory growth is set by reduced energetic costs at low population density, but the absolute scope is set by resource availability and consumption rates. Clearly, further work is necessary to evaluate these hypotheses in the field.

Taken together, our results support an emerging "general model" of biotic factors that affect recruitment through the first summer for Atlantic salmon and similar stream salmonids. Building on earlier work on recruitment of stream salmonids (Le Cren 1973; Elliott 1989), this model predicts that total cohort strength is largely determined during the early critical period by predation (Ward et al. 2008*a*, 2008*b*), limited suitable habitat (Lobón-Cerviá and Rincón 2004; Nislow et al. 2004), and other factors that reduce foraging success (Kennedy et al. 2008). Thereafter, compensatory responses, constrained by habitat and prey availability (Ward et al. 2009), primarily affect mean individual traits (growth, condition) rather than abundance (Einum et al. 2006; Nislow et al. 2011). By using a tracer approach to estimate time-integrated consumption, we were able to evaluate for the first time the energetic mechanisms underlying this transition from the critical period to subsequent compensatory performance for free-living fish.

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References

Blanchet, S., Bernatchez, L., and Dodson, J.J. 2007. Behavioural and growth responses of a territorial fish (Atlantic salmon, *Salmo salar*, L.) to multiple predatory cues. Ethology, **113**(11): 1061–1072. doi:10.1111/j.1439-0310.2007.01410.x.

Blanchet, S., Loot, G., and Dodson, J.J. 2008. Competition, predation and flow rate as mediators of direct and indirect effects in a stream food chain. Oecologia (Berl.), **157**(1): 93–104. doi:10.1007/s00442-008-1044-8. PMID:18465148.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49**(5): 1010–1017. doi:10.1139/f92-113.

Bränäs, E. 1995. First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. Evol. Ecol. **9**: 411–420. doi:10.1007/BF01237763.

Burnham, K.P., and Anderson, D.R. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol. Methods Res. **33**(2): 261–304. doi:10.1177/0049124104268644.

Carle, F.L., and Strub, M.R. 1978. A new method for estimating population size from removal data. Biometrics, **34**(4): 621–630. doi:10.2307/2530381.

Chen, C.Y., Stemberger, R.S., Klaue, B., Blum, J.D., Pickhardt, P.C., and Folt, C.L. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. Limnol. Oceanogr. **45**(7): 1525–1536. doi:10.4319/lo.2000.45.7.1525.

Chipps, S.R., and Wahl, D.H. 2008. Fish bioenergetics modeling in the 21st century: reviewing new insights and revisitng old constraints. Trans. Am. Fish. Soc. **137**(1): 298–313. doi:10.1577/T05-236.1.

Einum, S., Sundt-Hansen, L., and Nislow, K.H. 2006. The partitioning of density-dependent dispersal, growth and survival throughout ontogeny in a highly fecund organism. Oikos, **113**(3): 489–496. doi:10.1111/j.2006.0030-1299.14806.x.

Elliott, J.M. 1989. The critical period concept for juvenile survival and its relevance for population regulation in young sea trout, *Salmo trutta*. J. Fish Biol. **35**: 91–98. doi:10.1111/j.1095-8649.1989.tb03049.x.

Folt, C.L., Nislow, K.H., and Power, M.E. 1998. Implications of temporal and spatial scale for Atlantic salmon (*Salmo salar*) research. Can. J. Fish. Aquat. Sci. **55**(S1): 9–21. doi:10.1139/d98-017.

Forseth, T., Jonsson, B., Naeumann, R., and Ugedal, O. 1992. Radioisotope method for estimating food consumption by brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. **49**(7): 1328–1335. doi:10.1139/f92-148.

Gotceitas, V., and Godin, J.G.J. 1991. Foraging under the risk of predation in juvenile Atlantic salmon (*Salmo salar* L.): effects of social status and hunger. Behav. Ecol. Sociobiol. **29**(4): 255–261. doi:10.1007/BF00163982.

Grant, J.W.A., and Imre, I. 2005. Patterns of density-dependent growth in juvenile stream-dwelling salmonids. J. Fish Biol. **67**(Suppl. B): 100–110. doi:10.1111/j.0022-1112.2005.00916.x.

Henderson, J.N., and Letcher, B.H. 2003. Predation on stocked Atlantic salmon (*Salmo salar*) fry. Can. J. Fish. Aquat. Sci. **60**(1): 32–42. doi:10.1139/f03-001.

Hixon, M.A., and Beets, J.P. 1993. Predation, prey refuges, and the structure of coral reef fish assemblages. Ecol. Monogr. **63**(1): 77–101. doi:10.2307/2937124.

Hood, G.M. 2008. PopTools version 3.0.6 [computer program]. Available from http://www.cse.csiro.au/poptools [accessed 11 October 2008].

Horton, G.E., Letcher, B.H., Bailey, M.M., and Kinnison, M.T. 2009. Atlantic salmon (*Salmo salar*) smolt production: the relative importance of survival and body growth. Can. J. Fish. Aquat. Sci. **66**(3): 471–483. doi:10.1139/F09-005.

Imre, I., Grant, J.W.A., and Cunjak, R.A. 2005. Density-dependent growth of young-of-the-year Atlantic salmon *Salmo salar* in Catamaran Brook, New Brunswick. J. Anim. Ecol. **74**(3): 508–516. doi:10.1111/j.1365-2656.2005.00949.x.

Kaspersson, R., Hojesjo, J., and Pedersen, S. 2010. Effects of density on foraging success and aggression in age-structured groups of brown trout. Anim. Behav. **79**(3): 709–715. doi:10.1016/j.anbehav.2009.12.025.

Kennedy, B.P., Klaue, B., Blum, J.D., and Folt, C. 2004. Integrative measures of consumption rates in salmon: expansion and application of a trace element approach. J. Appl. Ecol. **41**(5): 1009–1020. doi:10.1111/j.0021-8901.2004.00956.x.

Kennedy, B.P., Nislow, K.H., and Folt, C.L. 2008. Habitat-mediated foraging limitations drive survival bottlenecks for juvenile salmon. Ecology, **89**(9): 2529–2541. doi:10.1890/06-1353.1. PMID:18831174.

Le Cren, E.D. 1973. The population dynamics of young trout (*Salmo*

trutta) in relation to density and territorial behavior. Rapp. P.-V. Reun.- Cons. Int. Explor. Mer, **164**: 241–246.

Leduc, A.O.H.C., Kelly, J.M., and Brown, G.E. 2004. Detection of conspecific alarm cues by juvenile salmonids under neutral and weakly acidic conditions: laboratory and field tests. Oecologia (Berl.), **139**(2): 318–324. doi:10.1007/s00442-004-1492-8. PMID: 14758533.

Lepak, J.M., Robinson, J.M., Kraft, C.E., and Josephson, D.C. 2009. Changes in mercury bioaccumulation in an apex predator in response to removal of an introduced competitor. Ecotoxicology, **18**(5): 488–498. doi:10.1007/s10646-009-0306-5. PMID:19277860.

Letcher, B.H., and Terrick, T.D. 1998. Maturation of male age-0 Atlantic salmon following a massive, localized flood. J. Fish Biol. **53**(6): 1243–1252. doi:10.1111/j.1095-8649.1998.tb00245.x.

Lima, S.L. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv. Stud. Behav. **27**: 215–290. doi:10.1016/S0065-3454(08)60366-6.

Lobón-Cerviá, J., and Rincón, P.A. 2004. Environmental determinants of recruitment and their influence on the population dynamics of stream-living brown trout *Salmo trutta*. Oikos, **105**(3): 641–646. doi:10.1111/j.0030-1299.2004.12989.x.

Metcalfe, N.B. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **55**(S1): 93–103. doi:10.1139/f98-005.

Milner, N.J., Elliott, J.M., Armstrong, J.D., Gardiner, R., Welton, J.S., and Ladle, M. 2003. The natural control of salmon and trout populations in streams. Fish. Res. **62**(2): 111–125. doi:10.1016/S0165-7836(02)00157-1.

Nislow, K.H., Folt, C., and Seandel, M. 1998. Food and foraging behavior in relation to microhabitat use and survival of age-0 Atlantic salmon. Can. J. Fish. Aquat. Sci. **55**(1): 116–127. doi:10.1139/f97-222.

Nislow, K.H., Folt, C.L., and Parrish, D.L. 1999. Favorable foraging locations for young Atlantic salmon: application to habitat and population restoration. Ecol. Appl. **9**(3): 1085–1099. doi:10.1890/1051-0761(1999)009[1085:FFLFYA]2.0.CO;2.

Nislow, K.H., Einum, S., and Folt, C. 2004. Testing predictions of the critical period for survival concept using experiments with stocked Atlantic salmon. J. Fish Biol. **65**(Suppl. s1): 188–200. doi:10.1111/j.0022-1112.2004.00561.x.

Nislow, K.H., Armstrong, J.D., and Grant, J.W.A. 2011. The role of competition in the ecology of juvenile Atlantic salmon. *In* Atlantic salmon Ecology. *Edited by* Ø. Aas, S. Einum, A. Klemetsen, and J. Skurdal. Wiley-Blackwell, Oxford, UK. pp. 171–190.

Orpwood, J.E., Griffiths, S.W., and Armstrong, J.D. 2006. Effects of food availability on temporal activity patterns and growth of Atlantic salmon. J. Anim. Ecol. **75**(3): 677–685. doi:10.1111/j.1365-2656.2006.01088.x. PMID:16689950.

Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B., Orrock, J.L., Peacor, S.D., Preisser, E.L., Schmitz, O.J., and Trussell, G.C. 2008. Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology, **89**(9): 2416–2425. doi:10.1890/07-1131.1. PMID:18831163.

Relyea, R.A. 2002. The many faces of predation: how induction, selection, and thinning combine to alter prey phenotypes. Ecology, **83**(7): 1953–1964. doi:10.1890/0012-9658(2002)083[1953:TMFOPH]2.0.CO;2.

Rennie, M.D., Collins, N.C., Shuter, B.J., Rajotte, J.W., and Couture, P. 2005. A comparison of methods for estimating activity costs of wild fish populations: more active fish observed to grow slower. Can. J. Fish. Aquat. Sci. **62**(4): 767–780. doi:10.1139/f05-052.

Rennie, M.D., Purchase, C.F., Lester, N., Collins, N.C., Shuter, B.J., and Abrams, P.A. 2008. Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. J. Anim. Ecol. **77**(5): 916–926. doi:10.1111/j.1365-2656.2008.01412.x. PMID:18557958.

Rowan, D.J., and Rasmussen, J.B. 1996. Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (Cs-137). Can. J. Fish. Aquat. Sci. **53**(4): 734–745. doi:10.1139/f95-046.

Taylor, B.W., McIntosh, A.R., and Peckarsky, B.L. 2001. Sampling stream invertebrates using electroshocking techniques: implications for basic and applied research. Can. J. Fish. Aquat. Sci. **58**(3): 437–445. doi:10.1139/f00-255.

Taylor, V.F., Jackson, B.P., and Chen, C.Y. 2008. Mercury speciation and total trace element determination of low-biomass biological samples. Anal. Bioanal. Chem. **392**(7–8): 1283–1290. doi:10.1007/s00216-008-2403-3. PMID:18828006.

Trudel, M., and Rasmussen, J.B. 1997. Modeling the elimination of mercury by fish. Environ. Sci. Technol. **31**(6): 1716–1722. doi:10.1021/es960609l.

Trudel, M., Tremblay, A., Schetagne, R., and Rasmussen, J.B. 2000. Estimating food consumption rates of fish using a mercury mass balance model. Can. J. Fish. Aquat. Sci. **57**(2): 414–428. doi:10.1139/f99-262.

Trudel, M., Tremblay, A., Schetagne, R., and Rasmussen, J.B. 2001. Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). Can. J. Fish. Aquat. Sci. **58**(2): 394–405. doi:10.1139/f00-252.

Vincenzi, S., Crivelli, A.J., Jesensek, D., and De Leo, G.A. 2008. The role of density-dependent individual growth in the persistence of freshwater salmonid populations. Oecologia (Berl.), **156**(3): 523–534. doi:10.1007/s00442-008-1012-3. PMID:18386068.

Walters, C.J., and Juanes, F. 1993. Recruitment limitation as a consequence of natural selection for use of restricted feeding habitats and predation risk taking by juvenile fishes. Can. J. Fish. Aquat. Sci. **50**(10): 2058–2070. doi:10.1139/f93-229.

Ward, D.M., and Hvidsten, N.A. 2010. Predation: compensation and context dependence. *In* Atlantic salmon ecology. *Edited by* Ø. Aas, A. Klemetsen, S. Einum, and J. Skurdal. Wiley-Blackwell, Oxford, UK. pp. 199–220.

Ward, D.M., Nislow, K.H., Armstrong, J.D., Einum, S., and Folt, C. L. 2007. Is the shape of the density–growth relationship for stream salmonids evidence for exploitative rather than interference competition? J. Anim. Ecol. **76**(1): 135–138. doi:10.1111/j.1365-2656.2006.01169.x. PMID:17184361.

Ward, D.M., Nislow, K.H., and Folt, C. 2008*a*. Do native species limit survival of reintroduced Atlantic salmon in historic rearing streams? Biol. Conserv. **141**(1): 146–152. doi:10.1016/j.biocon.2007.09.006.

Ward, D.M., Nislow, K.H., and Folt, C.L. 2008*b*. Predators reverse the direction of density dependence for juvenile salmon mortality. Oecologia (Berl.), **156**(3): 515–522. doi:10.1007/s00442-008-1011-4. PMID:18317816.

Ward, D.M., Nislow, K.H., and Folt, C.L. 2009. Increased population density and suppressed prey biomass: relative impacts on juvenile Atlantic salmon growth. Trans. Am. Fish. Soc. **138**(1): 135–143. doi:10.1577/T08-128.1.

Ward, D.M., Nislow, K.H., Chen, C.Y., and Folt, C.L. 2010*a*. Rapid, efficient growth reduces mercury concentrations in stream-dwelling Atlantic salmon. Trans. Am. Fish. Soc. **139**(1): 1–10. doi:10.1577/T09-032.1. PMID:20436784.

Ward, D.M., Nislow, K.H., Chen, C.Y., and Folt, C.L. 2010*b*.

Reduced trace element concentrations in fast-growing juvenile Atlantic salmon in natural streams. Environ. Sci. Technol. **44**(9): 3245–3251. doi:10.1021/es902639a. PMID:20356034.

Ward, D.M., Nislow, K.H., and Folt, C.L. 2010c. Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. Ann. N. Y. Acad. Sci. **1195**(1): 62–83. doi:10.1111/j.1749-6632.2010.05456.x. PMID: 20536817.

Werner, E.E., and Peacor, S.D. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology, **84**(5): 1083–1100. doi:10.1890/0012-9658(2003)084[1083:AROTII]2.0.CO;2.

Contrasting patterns of territoriality and foraging mode in two stream-dwelling salmonids, Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*)

Guðmundur Smári Gunnarsson and Stefán Óli Steingrímsson

Abstract: Territoriality and foraging behaviour play major roles in determining the abundance and distribution of mobile animals. To date, territorial behaviour of young-of-the-year (YOY) salmonids is typically described for sit-and-wait individuals that defend territories from one foraging station, but rarely for more mobile fish. We examined the territorial behaviour and foraging mode of 31 YOY Arctic char (*Salvelinus alpinus*) and 30 YOY brown trout (*Salmo trutta*) in relation to ecological factors in six rivers in northern Iceland. Arctic char used larger territories than brown trout, corresponding with high and low mobility prior to attacking prey, respectively. Within species, more mobile fish also used larger territories. Territory size increased with body size and declined with increased food abundance as predicted, but surprisingly increased with rising intruder pressure. Finally, Arctic char territories overlapped more and were less exclusively defended than brown trout territories. This study shows that territories of mobile individuals may not always pertain to the same rules as single central-place territories and highlights that territorial behaviour, and its role in population regulation, may vary between salmonid species.

Résumé : La territorialité et le comportement de recherche de nourriture jouent des rôles majeurs dans la détermination de l'abondance et de la répartition des animaux mobiles. À ce jour, le comportement territorial des jeunes de l'année (YOY) chez les salmonidés est typiquement décrit comme celui d'individus qui restent sur place en attente, tout en défendant un territoire à partir d'une station de recherche de nourriture, mais rarement comme celui de poissons mobiles. Nous examinons le comportement territorial et le mode de recherche de nourriture de 31 ombles chevaliers (*Salvelinus alpinus*) YOY et 30 truites brunes (*Salmo trutta*) YOY en fonction des facteurs écologiques dans six rivières du nord de l'Islande. Les ombles chevaliers utilisent des territoires plus grands que ceux des truites brunes, ce qui correspond à respectivement une forte et une faible mobilité avant l'attaque des proies. Au sein de chaque espèce, les poissons plus mobiles utilisent aussi de plus grands territoires. Comme prévu, la grandeur du territoire augmente aussi en fonction de la taille corporelle et décline lorsque l'abondance de nourriture augmente; de façon inattendue, elle s'accroît avec l'augmentation de la pression exercée par les intrus. Finalement, les territoires des ombles chevaliers se chevauchent plus et sont défendus de façon moins exclusive que ceux des truites brunes. Notre étude démontre que les territoires des individus mobiles peuvent ne pas être soumis aux mêmes règles que les territoires organisés autour d'un seul emplacement central; de plus, elle souligne que le comportement territorial, et son rôle dans la régulation des populations, peut varier d'une espèce de salmonidés à une autre.

[Traduit par la Rédaction]

Introduction

Animals may increase their fitness by defending resources such as food, shelter, and mates (Davies and Houston 1984). Such aggressive behaviour can result in competition for space (i.e., territories), which provides access to these resources and may play a role in determining population abundance (Patterson 1980; Newton 1992; López-Sepulcre and Kokko 2005). Importantly, the size and defence of territories (i.e., defended areas, sensu Noble 1939), and their effect on population regulation, depends on a range of ecological factors. For instance, territory size typically increases with body size (Schoener 1983), but decreases when food availability and consumer density increase in a variety of taxa (Myers et al. 1979; Dill et al. 1981; Loveridge et al. 2009). Although territory size is reported in many studies, details about how areas

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are exploited and defended are less often included (but see Stamps and Krishnan 1998; Meadows 2001).

Insights on how individuals exploit territories can be gained from the foraging mode literature. Pianka (1966) introduced two common foraging modes among mobile animals, the "widely forage" and the "sit-and-wait" modes (see also: Huey and Pianka 1981). Although Pianka (1983) assumed that sit-and-wait individuals had smaller home ranges than those that forage widely, empirical data connecting home range size and foraging mode are scarce (but see Katano 1996; Verwaijen and Van Damme 2008) and in some cases contradict these assumptions (Steingrímsson and Grant 2011). In general, studying territory size and foraging mode concurrently should yield a more comprehensive understanding of how animals respond to different ecological conditions.

Territorial behaviour of juvenile salmonids in streams (Kalleberg 1958) is typically described via the central-place territorial model (CPTM), which assumes that sit-and-wait individuals forage and attack intruders from, and return to, a single foraging station (Grant et al. 1989; Elliott 1990; but see Keeley and Grant 1995). Young-of-the-year (YOY) salmonids, however, often use alternative behavioural tactics, such as mobile foraging, where individuals actively search for prey (Grant and Noakes 1987; Nielsen 1992; Nakano et al. 1999), and multiple central-place foraging, where individuals sit and wait for prey but switch among several foraging stations (i.e., multiple CPTM; see Steingrímsson and Grant 2008). To date, these mobile individuals are rarely included in studies that map the space use of YOY salmonids (but see Nakano 1995 for older fish), even though these fish may patrol and defend large multiple central-place territories (Steingrímsson and Grant 2008, 2011). Hence, current knowledge on how territory size of YOY salmonids varies across ecological situations is biased towards sedentary individuals defending single central-place territories at high densities.

The effects of ecological factors on single central-place territories of YOY salmonids are well documented. Larger individuals defend larger territories presumably to encounter enough food to meet their metabolic demands (Grant and Kramer 1990), whereas territory size decreases with increased food abundance for similar reasons, i.e., fish maintain their food intake on a smaller territory at high food abundance (Keeley and Grant 1995; Keeley and McPhail 1998). Territories may also decrease with increased intruder pressure (Keeley 2000), and where visibility is low, for example, owing to decreased light intensity (Valdimarsson and Metcalfe 2001) or large substrate on the riverbed (Kalleberg 1958; Imre et al. 2002). In terms of foraging mode, which may be genetically or environmentally induced, fish may differ between species (Ferguson and Noakes 1982) or between conspecific individuals found in lakes and streams (Biro and Ridgway 1995) or in areas of faster and slower current in the same stream (Grant and Noakes 1987). Typically, fish are more mobile where drifting prey are scarce (e.g., in slow water) but use the sit-and-wait mode where drift is abundant (e.g., in faster water) (Grant and Noakes 1987; Nakano et al. 1999). Based on the few available studies, fish that forage from several stations may use large territories, with territory size being affected by dominance status (Nakano 1995), current velocity, prey availability (Steingrímsson and Grant 2011), and substrate size (Venter et al. 2008), but not necessarily being associated with body size or mobility (Steingrímsson and Grant 2008, 2011).

In this study we compared territorial and foraging behaviour of two salmonid species, Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*), and examined how these behaviours relate to ecological conditions in streams. First, because Arctic char is generally found in less productive and slower-running water than brown trout (Heggenes and Saltveit 2007; Tunney 2008), we predicted that Arctic char should use larger territories and be more mobile while foraging. Second, we predicted that more mobile fish of both species should use larger territories. Third, we predicted that territory size should increase with body size but decrease with increased food abundance, intruder pressure, and current velocity. Finally, we compared the exclusivity of Arctic char and brown trout territories by examining (*i*) the distribution of aggressive acts within the territory (Steingrímsson and Grant 2008) and (*ii*) the percent habitat saturation (sensu Grant and Kramer 1990), which indicates the degree to which territories overlap.

Materials and methods

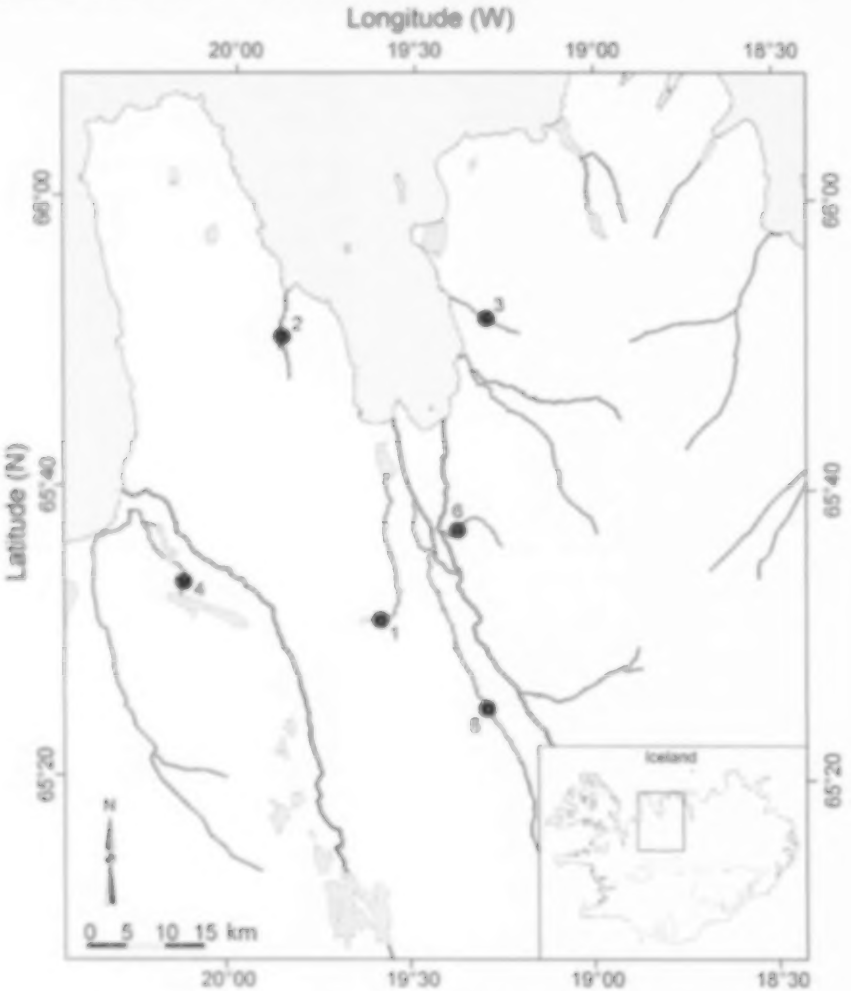
General information

Wild populations of Arctic char and brown trout were studied in six rivers in northern Iceland (Fig. 1), three rivers for each species, from June to August 2007 and 2008. In total, 31 YOY Arctic char and 30 YOY brown trout were observed (10–11 fish in each river). We selected rivers, and sites within rivers, to ensure that fish were observed under highly variable conditions in terms of physical characteristics and stream productivity. Thus, we searched for potential study sites and individuals in each river via snorkeling or by walking along the riverbanks and also included side channels and smaller tributaries. Only one salmonid species (i.e., the focal species) was detected in the study sites during the observation periods.

In each river, we caught fish with dip nets, either while snorkelling or from the riverbank, and anesthetized them briefly in a phenoxyethanol solution. Fork length was measured using calipers and reported to the nearest 0.1 cm. We tagged fish individually by injecting fluorescent elastomer paint (colours: red, green, or orange; Northwest Marine Technology, Inc., Shaw Island, Wash., USA) in two of six potential tagging locations on each fish (Steingrímsson and Grant 2003, 2008). When the fish had recovered from anaesthesia, we returned it in a dip net to the exact location of capture. No fish was observed for behaviour until at least 1 day after tagging.

When fish were continuously active and thus easily tracked over time, we sometimes observed untagged fish (26 of the 61 focal fish). For untagged Arctic char (14 fish) we drew a picture of the individual for identification to avoid confusing it with other fish; this was possible because of often distinct and easily recognizable parr marks in YOY Arctic char. Untagged brown trout (12 fish) are harder to recognize, and if we lost sight of the focal fish and there were any doubts about its identity upon reappearance, the observation period was cancelled. Sometimes, untagged fish were not caught after an observation period (nine Arctic char and six brown trout). In these cases, body size was visually estimated to the

Fig. 1. Location of the six study rivers in northwest Iceland. The three rivers for the Arctic char populations are (*i*) Myllulækur, (*ii*) Laxá í Skefilsstaðahreppi, and (*iii*) Deildará. The rivers for the brown trout populations are (4) Fremri-Laxá, (5) Húseyjarkvísl, (6) Þverá. The small map in the bottom-right corner shows the location of the study area within Iceland.



nearest 0.1 cm with regards to measured benthic landmarks (e.g., substrate).

Behavioural observations

Observations were all conducted during daylight (between 1023 and 1904 h) by G.S. Gunnarsson, either by recording behaviour directly on waterproof mylar sheets (28 fish) or later from videos recorded by G.S. Gunnarsson or S.Ó. Steingrímsson (33 fish). Each fish was monitored for approximately 40 min (range = 37–43 min), either from the stream bank (on mylar or video) or by snorkeling (mylar). Observations started no earlier than 10 min after fish identification to ensure that we would not affect their behaviour (see similar methods in Steingrímsson and Grant 2008). During the 40 min period, we recorded aggressive and foraging behaviour by documenting the direction (1–12 o'clock, 12 being upstream) and the radius (body lengths) of each observed act (sensu Grant et al. 1989). We also noted whether the focal fish fed from the river substrate, water column, or water surface and whether it started the prey attack from a sit-and-wait position or while moving. We classified a foraging attempt as mobile if a fish had moved continuously more than one body length immediately prior to attacking the prey, but as sedentary (i.e., sit-and-wait) if fish moved one body length or less before attacking prey. After the 40 min observation period, we counted foraging attempts for an additional 1–5 min to estimate foraging rate.

We facilitated the mapping of territories by placing a coordinate grid in each study site, at least 1 day before an observation period. The grid was composed of several 1 m long black metal poles (width = 8 mm), marked with tape at every 10 cm, which were placed on the river substrate, parallel and perpendicular to the stream flow. If the focal fish did not use distinct foraging stations, we recorded the location of each foraging attempt and aggressive act based on its position (i.e., *x*, *y* coordinates) within the grid. If the focal fish used

fixed foraging stations, the location of each station was marked on a map drawn of the river substrate. Each station was measured (to the nearest 1 cm) using the coordinate grid or by stringing a measuring tape through the territory and measuring the distance from the tape to each foraging station (sensu Steingrímsson and Grant 2008). For fish using foraging stations, the *x*, *y* coordinate of each foraging attempt and aggressive act was thus estimated based on the location of each station and the vector of the attempt (radius and direction, see above). Twenty-two Arctic char were observed by using the coordinate grid, and 9 were mapped based on their foraging stations. Only 1 brown trout was mapped using the grid, 26 were mapped from the location of their foraging stations, and 3 were mapped by using both systems.

Habitat measurements

The habitat of each territory was measured for several important ecological variables. Current velocity was measured over three 5 s intervals for each fish at 40% depth from the bottom with a current meter (FLO-MATE model 2000, Marsh–McBirney Inc., Frederick, Md., USA). Water depth was measured to the nearest centimetre using a metre stick. Dominant substrate size (sensu DeGraaf and Bain 1986) was estimated for and averaged across four equal areas of a circle extending in a 50 cm radius from the center of the territory. Water temperature was measured with a thermometer to the nearest 0.1 °C. Intruder pressure was estimated by counting the number of fish in approximately a 1 m radius from the focal fish on a regular basis (mean = 5.9 times) over the 40 min observation period.

Food abundance was estimated by placing a 250 μm drift net (net opening = 25 cm × 40 cm) in the center of each territory for 20 min immediately after observing the focal fish. During sampling, current velocity was measured at three different locations (middle, right, and left) in the mouth of the drift net at 50% of the drift net depth. Drift samples were stored in 70% ethanol solution and processed in the laboratory. All food items in each sample were counted under a stereomicroscope and categorized into families. Food items too large for YOY fish to eat (based on the allometry of maximum prey size for Atlantic salmon (*Salmo salar*) (Keeley and Grant 1997) were removed and excluded from the count. Food abundance (i.e., invertebrate drift rate) was calculated as the number of food items per minute entering the drift net area. If the net opening was not fully submerged in the water during sampling, drift rate was prorated to the net height.

Spatial analysis

A digital map was created for the territory of each fish in ArcView GIS 3.2 (with the Animal Movement extension added; Hooge and Eichenlaub 2000). Territory size was estimated via the minimum convex polygon method (MCP) (Schoener 1981) using the location of every aggressive and foraging act ($MCP_{100\%}$). For a comparison, territory size was also estimated after removing 5% of spatial outliers for each territory ($MCP_{95\%}$). Because the two estimates were highly correlated for both species (Arctic char: $\log_{10}MCP_{100\%}$ (m^2) = 0.181 + 0.879%$\log_{10}MCP_{95\%}$ (m^2), n = 31, R^2 = 0.864, $p < 0.001$; brown trout: $\log_{10}MCP_{100\%}$ (m^2) = 0.046 + 0.805$\log_{10}MCP_{95\%}$ (m^2), n = 30, R^2 = 0.889, $p < 0.001$), and both estimates were related to ecological correlates in a similar manner, only $MCP_{100\%}$ values are reported for the territory size analyses. For each aggressive individual, we also estimated a central-place territory size (hereafter "aggressive area") by superimposing the radii of all aggressive acts onto a single hypothetical station (i.e., (mean aggressive radius)^2 × π). This "circular method" is often used to map single central-place territories (e.g., Keeley and Grant 1995; Keeley 2000), but here indicates the central-place area defended at each station throughout the territory.

We used two methods to test whether territorial defence differed between species. First, we examined the distribution of aggressive acts directed toward conspecifics within the territory. To do this, the MCP boundary for all foraging attempts was drawn for 100%, 75%, and 50% foraging area (MCP_{FOOD}) by removing 0%, 25%, and 50% of the spatial outliers, respectively. We then estimated the proportion of aggressive acts outside each MCP_{FOOD} for each individual by counting aggressive acts inside and outside the respective MCP_{FOOD} boundary (Steingrímsson and Grant 2008). Second, we estimated percent habitat saturation (PHS) to examine territory overlap. For each individual, PHS was calculated as PHS = (D_c/D_{max}) × 100, where D_c is the counted fish density per square metre and D_{max} is the calculated maximum density per square metre, assuming all neighbouring fish defended a territory of the same size as the focal fish, i.e., maximum density (fish·m^{-2}) = 1 / territory size (m^2) (modified from Grant and Kramer 1990). A PHS that exceeds 100% suggests that territories overlap or that fish may not defend specific areas.

Statistical analysis

We tested for the effect of ecological variables on territory size in three ways. First, we tested whether the regression slope for territory size on each predictor differed between the two species by looking for an interaction between the two variables (analysis of covariance $(ANCOVA)_{species \times ecological\ variable}$). Second, we tested for the effect of all predictors simultaneously by establishing a multivariate general linear model with territory size as a dependent variable and species (categorical factor), fork length, drift rate, current velocity, intruder pressure, substrate size, and water depth as predictors. Here we report a model, established via backwards stepwise regression, excluding variables with $p > 0.15$. Importantly, the same predictors contributed to the model irrespective of whether nonsignificant variables were included or not. Furthermore, this model (out of all possible model combinations) had the lowest Akaike's information criterion (AIC_c) score, supporting our model selection (Motulsky and Christopoulos 2004). Third, we tested for the effect of each significant predictor separately for each species. Variables that were not normally distributed were either $\log_{10}$ or square-root transformed (Sokal and Rohlf 1994). Because the proportion of mobile foraging attempts equaled zero for some fish, 0.01 was added to the original value to allow for $\log_{10}$ transformation.

Results

Territory size and foraging behaviour

For YOY Arctic char, we recorded the location of 4077 foraging attempts and 169 aggressive acts for 31 fish (mean fork length = 4.4 cm) during a total of 1244 min of observa-

tion. For YOY brown trout, we recorded the location of 6870 foraging attempts and 83 aggressive acts for 30 fish (mean fork length = 4.0 cm) over 1200 min.

Territory size ($MCP_{100\%}$) was on average 4.4 times larger for Arctic char (mean = 0.558 m^2) than for brown trout (mean = 0.127 m^2) (Table 1; Fig. 2; $\log_{10}MCP_{100\%}$: Student's t test: $t = 5.346$, df = 59, $p < 0.001$). These patterns also emerged across populations; mean territory size was consistently larger for Arctic char populations (range = 0.287–0.780 m^2) than for brown trout populations (range = 0.089–0.177 m^2) (Fig. 3). Importantly, $MCP_{100\%}$ territories for Arctic char and brown trout were on average 8.3 and 2.3 times larger than for their respective aggressive areas, calculated based on the mean aggressive radius, assuming circular territories where all aggressive acts originate from one central-place (see Materials and methods) (Table 1). These aggressive areas, however, did not differ significantly in size between the two species (Student's t test: $t = 0.534$, df = 38, $p = 0.590$).

As predicted, more mobile fish used larger territories than sit-and-wait individuals. First, at the species level, Arctic char used larger territories than brown trout and were also more frequently mobile prior to attacking prey (25% vs. 2%, respectively) (Table 1; $\log_{10}$ proportion mobile foraging attempts + 0.01; Student's t test: $t = 5.541$, df = 55, $p < 0.001$). Second, at the individual level, territory size increased with increasing proportion of mobile foraging attempts for both species (Fig. 4). In contrast, the two species did not differ in the distance at which they attacked food (Table 1; $\log_{10}$ foraging radius: Student's t test, $t = -0.194$, df = 59, $p = 0.847$). Also, although territory size increased with foraging radius among brown trout ($\log_{10}$ territory size (m^2) = 2.375 $\log_{10}$ foraging radius (no. body lengths) – 1.234, $n = 30$, $p = 0.013$), no such relationship was detected for Arctic char ($p = 0.727$).

Arctic char and brown trout fed predominantly on drifting invertebrates. Arctic char attacked prey 6.1 times·min^{-1} on average (Table 1), of which 92.5% (range: 38.1%–100%) of attempts were directed towards the water column, 4.1% (range: 0.0%–63.4%) towards the water surface, and only 3.7% (range: 0.0%–35.2%) towards benthic prey. Similarly, brown trout attacked prey 8.4 times·min^{-1} (Table 1), of which 92.1% (range: 55.6%–100%), 1.7% (range: 0.0%–8.7%), and 6.3% (range: 0.0%–39.4%) were directed towards the water column, water surface, and the river substrate, respectively.

Territory size and ecological factors

Territorial behaviour of Arctic char and brown trout was observed over a large range of environmental and ecological conditions (Table 2). A multivariate analysis revealed that variation in territory size was best explained by species differences and three ecological variables: fork length, food abundance, and intruder pressure (Table 3). First, territories of YOY Arctic char were larger than those of YOY brown trout, even after removing the effects of fork length, food abundance, and intruder pressure (Table 3; $p = 0.002$). Second, as predicted, territory size increased with fork length (Table 3; $p = 0.003$). Although the slope of this relationship did not differ between the two species ($ANCOVA_{species \times fork\ length}$: $F_{[1,57]} = 0.361$, $p = 0.551$), separate analyses showed that territories increased significantly with body size for brown trout, but not for Arctic char (Fig. 5*a*). Third, as expected, territory size decreased with increased food abundance (Table 3; $p = 0.002$). The slope of this relationship did not differ significantly between the two species ($ANCOVA_{species \times food\ abundance}$: $F_{[1,57]} = 0.58$, $p = 0.449$), although it was significant for Arctic char but not for brown trout when analysed separately (Fig. 5*b*). Fourth, unexpectedly, territory size increased with intruder pressure (Table 3; $p = 0.010$). Again, the slope did not differ between the two species ($ANCOVA_{species \times intruder\ pressure}$: $F_{[1,57]} = 1.23$, $p = 0.271$), but when analysed separately, territory size increased with intruder pressure in brown trout but not in Arctic char (Fig. 5*c*). Finally, a multivariate analysis revealed no significant effects of current velocity, water depth, and substrate size on territory size (Table 3).

Patterns of territorial defence

In this study, aggression was detected for 21 of 31 Arctic char and 20 of 30 brown trout. Interestingly, territories were significantly larger for aggressive brown trout than for those where aggression was not detected (Student's t test, $t = 2.391$, df = 28, $p = 0.033$), whereas no difference was detected for Arctic char (Student's t test, $t = 0.093$, df = 29, $p = 0.928$). The rate of aggression did not differ between the two species (Table 1; Mann–Whitney: $Z = 0.63$, $n = 41$, $p = 0.514$), while it increased with increasing intruder pressure in Arctic char (Spearman's $r = 0.417$, $n = 26$, $p = 0.034$), but not significantly so in brown trout (Spearman's $r = 0.342$, $n = 27$, $p = 0.081$).

The proportion of aggressive acts found outside the 50% MCP_{FOOD} area (minimum convex polygon with 50% foraging outliers removed) differed between the two species (Mann–Whitney: $Z = 4.8$, $n = 41$, $p < 0.001$), indicating that Arctic char attacked more intruders (20%) than brown trout (7%) deep within their territory. Alternatively, the proportion of aggressive acts outside the 100% MCP_{FOOD} (Arctic char: 34%; brown trout: 31%) and 75% MCP_{FOOD} (Arctic char: 71%; brown trout: 72%) did not vary between the two species (100% MCP_{FOOD}: Mann–Whitney: $Z = 0.21$, $n = 41$, $p = 0.829$ and 75% MCP_{FOOD}: Mann–Whitney: $Z = 0.08$, $n = 41$, $p = 0.935$).

Mean PHS, which indicates how much of the local habitat of each focal fish is occupied by territories, was significantly greater for Arctic char (85.6%) than for brown trout (20.8%) ($\log_{10}$ PHS; Student's t test: $t = 4.178$, df = 59, $p < 0.001$) (Fig. 6). These species differences in PHS were also reflected in the number of fish experiencing overlapping territories; i.e., PHS exceeded 100% for 10 Arctic char (maximum: 312%), but exceeded 100% for only 1 brown trout (maximum: 104%). Hence, Arctic char appear to share their local habitat to a much higher degree than brown trout.

Discussion

Variation in territory size and foraging behaviour

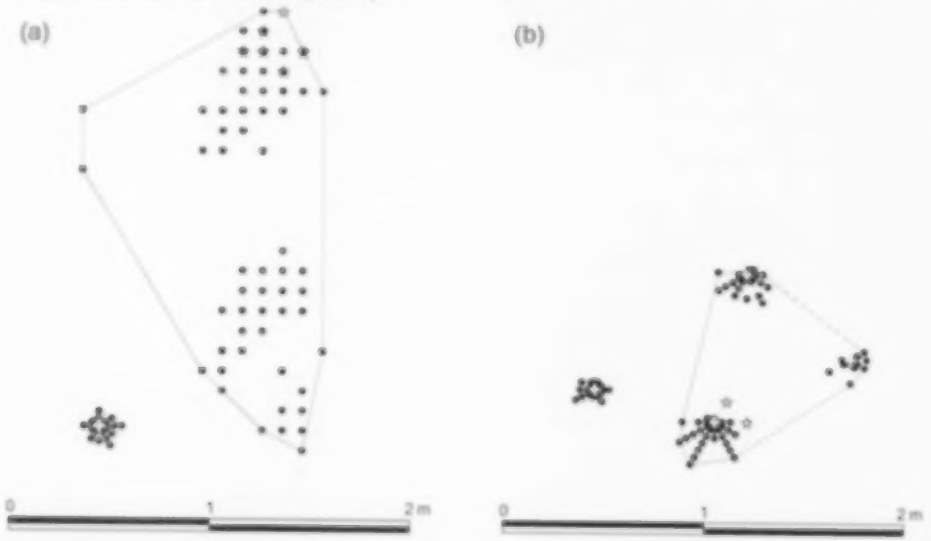
Territories mapped via the central-place territorial model (i.e., CPTM) are very useful for predicting the minimum space-use requirements of stream salmonids and their population consequences (Grant and Kramer 1990). These traditional estimates, however, may not always accurately reflect the size or the exploitation of the area used (see Steingríms-

Table 1. Summary of behavioural characteristics for 31 Arctic char and 30 brown trout from six study rivers in northwest Iceland.

	Arctic char		Brown trout	
Variable	Mean	Range	Mean	Range
Territory size ($MCP_{100\%}$, m^2)	0.558	0.015–1.725	0.127	0.012–0.542
95% territory size ($MCP_{95\%}$, m^2)	0.365	0.008–1.660	0.069	0.005–0.477
Foraging rate (attacks·min^{-1})	6.1	1.8–14.0	8.4	2.8–13.8
Aggression rate (attacks·min^{-1})	0.13	0.00–0.55	0.06	0.00–0.28
Proportion of mobile foraging attempts	0.25	0.00–0.91	0.02	0.00–0.07
Foraging radius (no. body lengths)	1.22	0.76–1.93	1.22	0.75–1.51
Aggressive radius (no. body lengths)	3.17	1.00–5.60	3.10	1.67–5.00
Aggressive area (m^2)[a]	0.068	0.005–0.195	0.055	0.012–0.146

[a]Aggressive area = (mean aggressive radius)$^2 \times \pi$ and was calculated for the 21 Arctic char and 20 brown trout where aggressive behaviour was observed.

Fig. 2. Variability in territory size for 31 Arctic char and 30 brown trout in six Icelandic rivers. The maps show the smallest and biggest territories for Arctic char (*a*) and brown trout (*b*), where the location of foraging attempts, aggressive acts, and foraging stations are represented by a solid circle, a star, and a grey cross, respectively.



son and Grant 2008). In terms of size, this study yielded larger brown trout territories (mean = 0.127 m^2) than the single central-place estimates from Elliott (1990) (0.049 m^2; for 4 cm long fish, as in this study) or when our data were mapped via the circular method (mean aggressive area = 0.055 m^2). For Arctic char, territory size based on the relative location of all foraging and aggressive acts was also larger (mean = 0.558 m^2) than the aggressive area mapped around each central place (mean = 0.067 m^2). Similarly, YOY Atlantic salmon territories mapped for fish using multiple central places (Steingrímsson and Grant 2008; Venter et al. 2008) were larger than earlier estimates for Atlantic salmon (Kalleberg 1958; Keeley and Grant 1995).

Studies that map territories from one foraging station (Elliott 1990; Keeley 2000) may also yield a biased view of territory use and foraging costs because they focus on sedentary fish at one end of the foraging mode spectrum (Steingrímsson and Grant 2011). In this study, however, we sampled tagged fish irrespective of mobility. As predicted, Arctic char used larger areas than brown trout and were more mobile prior to attacking prey (Tunney 2008); a similar trend has been seen among species of lizards (Verwaijen and Van Damme 2008). Furthermore, within species, more mobile (i.e., widely foraging) fish used larger areas (Katano 1996); this contradicts Steingrímsson and Grant's (2011) study, where the most mobile YOY Atlantic salmon did not occupy the largest multiple central-place territories. For our study fish, foraging radius plays a less obvious role than mobility in determining territory size; brown trout territories were positively related to foraging radius, whereas Arctic char territories were not. Finally, although salmonids in less productive waters may forage more on benthos (Nakano et al. 1999), Arctic char fed as much on drifting prey (92.5%) as did brown trout (92.1%) and even Atlantic salmon (>99%, Keeley and Grant 1995; 91.2%, Steingrímsson and Grant 2011).

In this study, brown trout typically used fixed foraging stations, whereas Arctic char more frequently moved around when foraging and less often returned to the station from which an attack was initiated (G.S. Gunnarsson, personal ob-

Fig. 3. Territory size for three study populations of each species, Arctic char and brown trout, in northwest Iceland. The boxes show the 25th and 75th percentile, whereas the line inside each box represents the median for each study river. Error bars above and below each box indicate the 90th and 10th percentiles, respectively, whereas the dots depict extreme values.

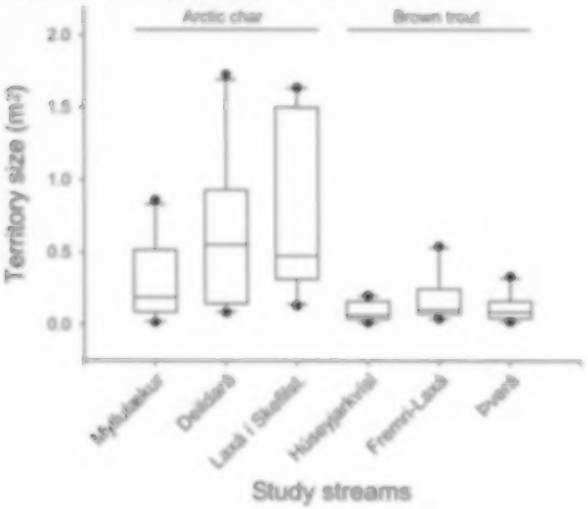
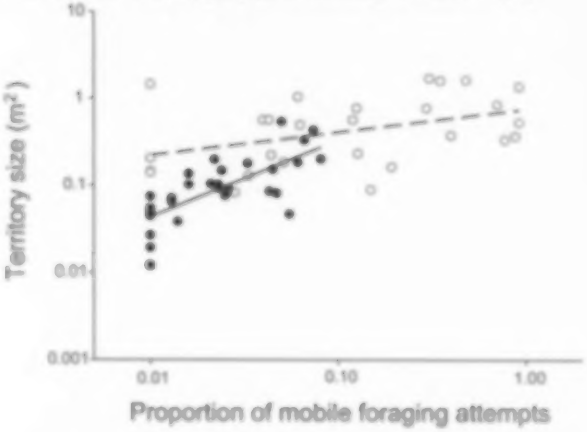


Fig. 4. The relationship between territory size and mobility for Arctic char (open circles and broken line) and brown trout (solid circles and line) in six rivers in northwest Iceland. The equations best describing these relationships are Arctic char: $\log_{10}$ territory size (m^2) = 0.350 $\log_{10}$ (proportion of mobile foraging attempts + 0.01) – 0.045, R^2 = 0.284, n = 27, p = 0.004; brown trout: $\log_{10}$ territory size (m^2) = 0.884 $\log_{10}$ (proportion of mobile foraging attempts + 0.01) + 0.406, R^2 = 0.538, n = 30, $p < 0.001$. A foraging attempt was classified as "mobile" if a fish moved continuously for more than one body length prior to initiating an attack on prey.



servation). Therefore, most brown trout could be mapped from one or several foraging stations, whereas Arctic char were in most cases mapped using a grid on the stream bottom. Similarly to Arctic char, a switch to more mobile foraging also occurs in a closely related species, brook trout (*Salvelinus fontinalis*) (Grant and Noakes 1987), whereas the use of fixed foraging stations by brown trout is more similar to Atlantic salmon (Keeley and Grant 1995; Steingrímsson and Grant 2008). These findings suggest systematic differences in territorial behaviour among salmonid genera, an issue that warrants further study.

Territory size and ecological factors

Territories of YOY Arctic char and YOY brown trout were affected by several ecological variables, which (*i*) either contradict or agree with earlier studies and (*ii*) highlight the differences between territories mapped via the CPTM (e.g., Keeley 2000) and those mapped by including more mobile fish (e.g., Steingrímsson and Grant 2008). First, as predicted, larger fish used larger territories. These findings agree with previous single central-place studies on YOY salmonids (e.g., Grant et al. 1989; Keeley and McPhail 1998; Keeley 2000). Interestingly, when analysed separately, brown trout showed an allometric increase in territory size, whereas Arctic char did not. Because Arctic char use larger territories than brown trout, large territories of widely foraging YOY salmonids and those using multiple central places (Steingrímsson and Grant 2008) may not be as strongly related to body size as single central-place territories.

Second, as predicted, territory size declined with increasing food abundance. These findings are consistent with several studies on YOY salmonids (Keeley and Grant 1995; Keeley 2000) and other fish species (Hixon 1981) and show that this relationship may also emerge when territories are mapped outside the CPTM (see also Steingrímsson and Grant 2011). Hence, increased territory size at low food abundance may not only be mediated through (*i*) increased foraging and aggressive radii at one foraging station (e.g., Elliott 1990; Keeley 2000), but also via (*ii*) longer distance travelled between stations of multiple central-place territories (Steingrímsson and Grant 2011) and (*iii*) increased foraging mobility (Arctic char). Finally, because territory size declined for Arctic char but not for brown trout, this study suggests that including territories of mobile fish may reveal species differences in the response to prey density not detected by the CPTM.

Third, this study yielded an unexpected positive relationship between intruder pressure and territory size, which was significant for brown trout but not for Arctic char. In this case a negative relationship was predicted because defence costs should increase with rising intruder pressure and make smaller territories more economical (Hixon 1980). However, although salmonid studies may report a negative relationship at high-density experimental conditions (e.g., Keeley 2000: 5.3, 10.2, and 21.1 fish·m^{-2}), these are not necessarily detected at lower population densities (e.g., Keeley and Grant 1995: mean: 3.2 fish·m^{-2}; Keeley and McPhail 1998: mean: 2.9 fish·m^{-2}). In this study, mean population density was relatively low at 1.50 and 1.46 fish·m^{-2} for Arctic char and brown trout, respectively, which may partly explain why territory size did not decrease with increased densities. This explanation, however, may apply better to brown trout, which experienced much lower habitat saturation (PHS = 20.8%) than Arctic char (PHS = 85.6%), because trout defended relatively small territories. Alternatively, because we followed fish irrespective of their social status, we may also, at high PHS, be observing subordinate fish that are repeatedly dis-

Table 2. Summary of ecological characteristics of the territories used by 31 Arctic char and 30 brown trout in six rivers in northwest Iceland.

	Arctic char		Brown trout	
Variable	Mean	Range	Mean	Range
Fork length (cm)	4.4	3.4–5.4	4.0	3.3–4.6
Drift rate (no. food items·min^{-1}·net^{-1})[a]	41.5	0.1–443.6	44.0	1.5–179.6
Intruder pressure (no. fish in a 1 m radius)	3.7	0.3–12.0	3.6	0.0–6.7
Substrate size[b]	3.8	1.0–6.0	3.3	1.0–5.0
Current velocity (cm·s^{-1})	5.2	0.0–15.0	15.3	0.7–35.0
Temperature (°C)	14.2	9.8–18.2	15.5	11.6–19.0
Water depth (cm)	35.9	18.0–60.0	23.0	5.0–36.0

[a]The area of the drift net opening was 25 cm × 40 cm; mesh size was 250 μm.

[b]The classification of the substrate was as follows: 1, clay and silt: <0.0625 mm; 2, sand: 0.0625–2 mm; 3, gravel: 2–16 mm; 4, pebble: 16–64 mm; 5, cobble: 64–256 mm; 6, bolder: >256 mm; 7, bedrock (see DeGraaf and Bain 1986).

Table 3. A multivariate model (analysis of covariance) testing for the influence of species and ecological predictors on territory size for 31 Arctic char and 30 brown trout in six rivers in northwest Iceland.

Variable[a]	Coefficient	SE	*F*	*df*	*p*
Final model					
Species	0.203	0.063	10.403	1,55	0.002
Fork length (cm)	3.464	1.128	9.424	1,55	0.003
Drift rate (no. food items·min^{-1}·net^{-1})[b]	–0.272	0.083	10.754	1,55	0.002
Intruder pressure (no. fish in a 1 m radius)	0.225	0.084	7.199	1,55	0.010
Current velocity (cm·s^{-1})	0.959	0.562	2.917	1,55	0.093
Substrate size[c,d]	–0.155		1.322	1,54	0.255
Water depth (cm)[d]	–0.066		0.234	1,53	0.631

Note: The model was selected using a stepwise backwards approach with species as a categorical factor. SE, standard error.

[a]Territory size, fork length, and drift rate were $\log_{10}$-transformed, whereas intruder pressure and current velocity were square-root transformed.

[b]The area of the drift net opening was 25 cm × 40 cm; mesh size was 250 μm.

[c]See Table 2 for the classification of substrate size.

[d]These variables were removed from the final model because $p > 0.15$. The final model also had the lowest AIC_c value (61.1). The models with the second-lowest (61.8) and third-lowest (62.2) AIC_c scores excluded (*i*) substrate size, water depth, and current velocity and (*ii*) only water depth, respectively.

placed and thus eventually end up using larger, less exclusively defended areas (G.S. Gunnarsson, personal observation; see Nakano 1995 for a similar trend).

Territorial defence

One historical consequence of mapping territories from one central-place station is that more mobile fish are dismissed as nonterritorial. Although this assumption likely holds in some situations (Elliott 1990), brown trout in this study exhibited more aggression when exploiting large territories. One way to estimate how territories are defended is to examine how aggression toward intruders is distributed within the area (Steingrímsson and Grant 2008). In this study, Arctic char attacked a higher proportion of intruders deep inside their territories than brown trout. Thus, brown trout may defend their territories more exclusively than Arctic char, which used larger areas that could be more costly to defend (Schoener 1983). However, the less exclusive defence of Arctic char territories compared with that of brown trout territories cannot entirely be explained by territory size. Territories of YOY Atlantic salmon are larger (mean = 0.932 m^2) than territories of both YOY Arctic char and YOY brown trout, but appear to be defended as exclusively as those for brown trout in this study (7.2% of attacks inside the 50% foraging area; Steingrímsson and Grant 2008). These results suggest different patterns of defence for Arctic char than for brown trout and Atlantic salmon, which use smaller and larger territories, respectively.

Another way to investigate how territories are defended is via PHS, which indicates the percentage of the river bottom occupied by territories. In this study, PHS was much higher for Arctic char (mean = 85.6%) than for brown trout (mean = 20.8%). More importantly, PHS exceeded 100% for 10 Arctic char (max = 312%), but exceeded 100% for only 1 brown trout (max = 104%). Hence, Arctic char defend their territories less exclusively than brown trout. At a high PHS, a negative relationship was expected between territory size and intruder pressure (see above); perhaps this did not apply to Arctic char because their territories are not exclusive at high densities. Finally, a high PHS for Arctic char may result from individuals defending three-dimensional territories throughout the water column and not being confined to the river bottom, especially in pools (Grant and Kramer 1990; see also Nakano 1995). In this study, Arctic char indeed used deeper waters than brown

Fig. 5. Territory size for 31 Arctic char (open circles and broken line) and 30 brown trout (solid circles and solid line) in six Icelandic rivers in relation to fork length (*a*), drift rate (*b*), and intruder pressure (*c*). The equations best describing the relationships are as follows: (*a*) Arctic char: $\log_{10}$ territory size (m^2) = 3.019 $\log_{10}$ fork length (cm) – 2.400, $R^2 = 0.111$, $n = 31$, $p = 0.067$); brown trout: $\log_{10}$ territory size (m^2) = 4.745 $\log_{10}$ fork length (cm) – 3.893, $R^2 = 0.149$, $n = 30$, $p = 0.035$. (*b*) Arctic char: $\log_{10}$ territory size (m^2) = –0.163 $\log_{10}$ drift rate (no.·min^{-1}·net^{-1}) – 0.353, $R^2 = 0.128$, $n = 31$, $p = 0.048$; brown trout: $\log_{10}$ territory size (m^2) = –0.025 $\log_{10}$ drift rate (no.·min^{-1}·net^{-1}) – 1.013, $R^2 = 0.001$, $n = 30$, $p = 0.857$. (*c*) Arctic char: $\log_{10}$ territory size (m^2) = 0.085 intruder pressure$^{1/2}$ – 0.615, $R^2 = 0.015$, $n = 31$, $p = 0.517$); brown trout: $\log_{10}$ territory size (m^2) = 0.286 intruder pressure$^{1/2}$ – 1.568, $R^2 = 0.181$, $n = 30$, $p = 0.019$). Log_{10}- and square-root transformed labels on both axes have been replaced with the original values. In (*b*), drift rate is calculated based on the area of the drift net opening (25 cm × 40 cm).

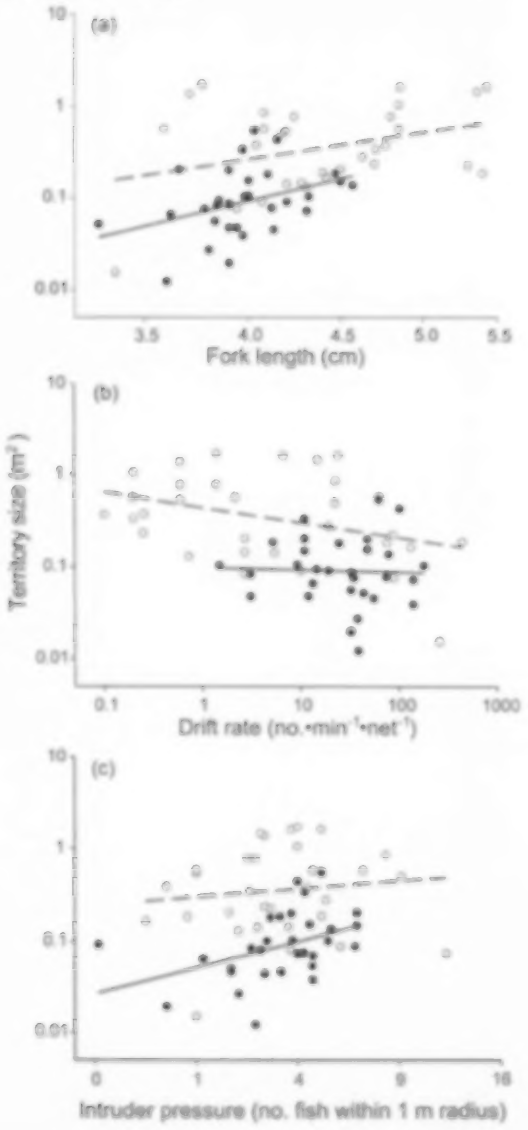
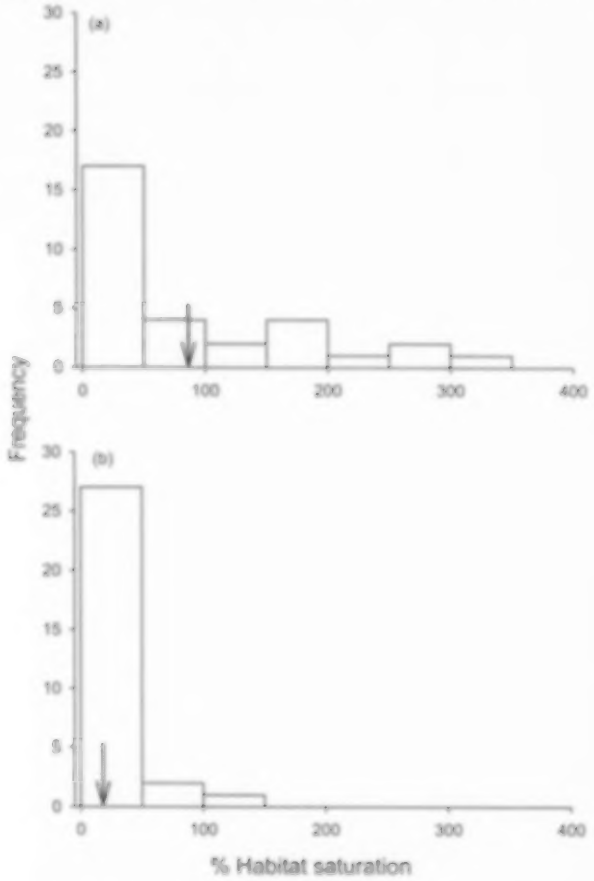


Fig. 6. Estimated percent habitat saturation (PHS) for young-of-the-year (YOY) Arctic char (*a*) and YOY brown trout (*b*) in each of three study rivers in northwest Iceland. PHS indicates the proportion of habitat covered by territories based on fish density and territory size for each individual. The arrows show the mean PHS for each species.



trout, which may partly explain their high PHS. In summary, although some brown trout and Arctic char may defend typical central-place territories, Arctic char appears to share local areas to a greater degree than most salmonids, either by not defending their whole territory or via stacking of territories in the water column.

Population ecology of stream-dwelling salmonids

Territorial behaviour plays a major role in the population regulation of stream-dwelling salmonids (Elliott 1990; Grant and Kramer 1990). To date, most studies on this topic have used the CPTM to estimate territory size, which in turn is a key predictor of important aspects of salmonid ecology, such as local population densities and density-dependent growth, emigration, and mortality (Elliott 1990; Grant and Kramer 1990). However, although single central-place territory estimates are good predictors of the minimum space-use requirements of stream salmonids (Grant and Kramer 1990), these estimates exclude fish that forage widely or use several foraging stations (Elliott 1990; but see Steingrímsson and Grant

2008). By mapping territories irrespective of foraging tactics, this study gave a more comprehensive view of territoriality in salmonids and revealed difference that would not be detected via the CPTM approach. Hence, this study showed that (*i*) Arctic char and brown trout used larger territories than is suggested by the CPTM, (*ii*) territory size was positively related to foraging mobility, both within and among species, (*iii*) these larger territories did not always associate with ecological factors in the same way as territories mapped via the CPTM (see also Steingrímsson and Grant 2008; 2011), and (*iv*) across the six study populations, brown trout defended territories more exclusively than Arctic char. In conclusion, by sampling fish over a wide range of mobility, future studies should provide a more comprehensive understanding of how salmonids differ in the way they exploit, defend, and share local habitats and how behaviour shapes individual fitness and population dynamics.

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References

Biro, P.A., and Ridgway, M.S. 1995. Individual variation in foraging movements in a lake population of young-of-the-year brook charr (*Salvelinus fontinalis*). Behaviour, **132**(1–2): 57–74. doi:10.1163/156853995X00289.

Davies, N.B., and Houston, A.I. 1984. Territory economics. *In* Behavioural ecology: an evolutionary approach. 2nd ed. *Edited by* J.R. Krebs and N.B. Davies. Blackwell, Oxford, UK. pp.148–169.

DeGraaf, D.A., and Bain, L.H. 1986. Habitat use by and preferences of juvenile Atlantic salmon in two Newfoundland rivers. Trans. Am. Fish. Soc. **115**(5): 671–681. doi:10.1577/1548-8659(1986)115<671:HUBAPO>2.0.CO;2.

Dill, L.M., Ydenberg, R.C., and Fraser, A.H.G. 1981. Food abundance and territory size in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. **59**(9): 1801–1809. doi:10.1139/z81-247.

Elliott, J.M. 1990. Mechanisms responsible for population regulation in young migratory trout *Salmo trutta*. III. The role of territorial behaviour. J. Anim. Ecol. **59**(3): 803–818. doi:10.2307/5015.

Ferguson, M.M., and Noakes, D.L.G. 1982. Genetics of social behaviour in charrs (*Salvelinus* species). Anim. Behav. **30**(1): 128–134. doi:10.1016/S0003-3472(82)80247-9.

Grant, J.W.A., and Kramer, D.L. 1990. Territory size as a predictor of the upper limit to population density of juvenile salmonids in streams. Can. J. Fish. Aquat. Sci. **47**(9): 1724–1737. doi:10.1139/f90-197.

Grant, J.W.A., and Noakes, D.L.G. 1987. Movers and stayers: foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. J. Anim. Ecol. **56**(3): 1001–1013. doi:10.2307/4962.

Grant, J.W.A., Noakes, D.L.G., and Jonas, K.M. 1989. Spatial distribution of defence and foraging in young-of-the-year brook charr, *Salvelinus fontinalis*. J. Anim. Ecol. **58**(3): 773–784. doi:10.2307/5123.

Heggenes, J., and Saltveit, S.J. 2007. Summer stream habitat partitioning by sympatric Arctic charr, Atlantic salmon and brown trout in two sub-arctic rivers. J. Fish Biol. **71**(4): 1069–1081. doi:10.1111/j.1095-8649.2007.01573.x.

Hixon, M.A. 1980. Food-production and competitor density as the determinants of feeding territory size. Am. Nat. **115**(4): 510–530. doi:10.1086/283577.

Hixon, M.A. 1981. An experimental analysis of territoriality in the California reef fish *Embiotoca jacksoni* (*Embiotocidae*). Copeia, **1981**(3): 653–665. doi:10.2307/1444571.

Hooge, P.N., and Eichenlaub, B. 2000. Animal movement extension to Arcview, version 2.0. Science Center - Biological Science Office, US Geological Survey, Anchorage, Alaska.

Huey, R.B., and Pianka, E.R. 1981. Ecological consequences of foraging mode. Ecology, **62**(4): 991–999. doi:10.2307/1936998.

Imre, I., Grant, J.W.A., and Keeley, E.R. 2002. The effect of visual isolation on territory size and population density of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **59**(2): 303–309. doi:10.1139/f02-010.

Kalleberg, H. 1958. Observation in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L., and *S. trutta* L.). Inst. Freshw. Res Drottningholm Rep. **39**: 55–98.

Katano, O. 1996. Foraging tactics and home range of dark chub in a Japanese river. Oecologia (Berl.), **106**(2): 199–205. doi:10.1007/BF00328599.

Keeley, E.R. 2000. An experimental analysis of territory size in juvenile steelhead trout. Anim. Behav. **59**(3): 477–490. doi:10.1006/anbe.1999.1288. PMID:10715169.

Keeley, E.R., and Grant, J.W.A. 1995. Allometric and environmental correlates of territory size in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **52**(1): 186–196. doi:10.1139/f95-019.

Keeley, E.R., and Grant, J.W.A. 1997. Allometry of diet selectivity in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **54**(8): 1894–1902. doi:10.1139/cjfas-54-8-1894.

Keeley, E.R., and McPhail, J.D. 1998. Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). Behaviour, **135**: 65–82.

López-Sepulcre, A., and Kokko, H. 2005. Territorial defense, territory size, and population regulation. Am. Nat. **166**(3): 317–329. doi:10.1086/432560. PMID:16224687.

Loveridge, A.J., Valeix, M., Davidson, Z., Murindagomo, F., Fritz, H., and Macdonald, D.W. 2009. Changes in home range size of African lions in relation to pride size and prey biomass in a semi-arid savanna. Ecography, **32**(6): 953–962.

Meadows, D.W. 2001. Centre-edge differences in behaviour, territory size and fitness in clusters of territorial damselfish: patterns, causes, and consequences. Behaviour, **138**(9): 1085–1116. doi:10.1163/156853901753287154.

Motulsky, H., and Christopoulos, A. 2004. Fitting models to biological data using linear and nonlinear regression: a practical guide to curve fitting. Oxford University Press, Oxford, UK.

Myers, J.P., Connors, P.G., and Pitelka, F.A. 1979. Territory size in wintering Sanderlings — effect of prey abundance and intruder density. Auk, **96**(3): 551–561.

Nakano, S. 1995. Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. J. Anim. Ecol. **64**(1): 75–84. doi:10.2307/5828.

Nakano, S., Fausch, K.D., and Kitano, S. 1999. Flexible niche

partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. J. Anim. Ecol. **68**(6): 1079–1092. doi:10.1046/j.1365-2656.1999.00355.x.

Newton, I. 1992. Experiments on the limitation of bird numbers by territorial behavior. Biol. Rev. Camb. Philos. Soc. **67**(2): 129–173. doi:10.1111/j.1469-185X.1992.tb01017.x.

Nielsen, J.L. 1992. Microhabitat-specific foraging behavior, diet, and growth of juvenile coho salmon. Trans. Am. Fish. Soc. **121**(5): 617–634. doi:10.1577/1548-8659(1992)121<0617:MFBDAG>2.3.CO;2.

Noble, G.K. 1939. The role of dominance in the life of birds. Auk, **56**: 253–273.

Patterson, I.J. 1980. Territorial behavior and the limitation of population-density. Ardea, **68**(1–4): 53–62.

Pianka, E.R. 1966. Convexity, desert lizards, and spatial heterogeneity. Ecology, **47**(6): 1055–1059. doi:10.2307/1935656.

Pianka, E.R. 1983. Evolutionary ecology. 3rd ed. Harper and Row, New York.

Schoener, T.W. 1981. An empirically based estimate of home range. Theor. Popul. Biol. **20**(3): 281–325. doi:10.1016/0040-5809(81)90049-6.

Schoener, T.W. 1983. Simple models of optimal territory size: a reconciliation. Am. Nat. **121**(5): 608–629. doi:10.1086/284090.

Sokal, R.R., and Rohlf, F.J. 1994. Biometry. 3rd ed. W.H. Freeman, San Francisco, Calif.

Stamps, J.A., and Krishnan, V.V. 1998. Territory acquisition in lizards. IV. Obtaining high status and exclusive home ranges. Anim. Behav. **55**(2): 461–472. doi:10.1006/anbe.1997.0612. PMID:9480712.

Steingrímsson, S.Ó., and Grant, J.W.A. 2003. Patterns and correlates of movement and site fidelity in individually tagged young-of-the-year Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **60**(2): 193–202. doi:10.1139/f03-012.

Steingrímsson, S.Ó., and Grant, J.W.A. 2008. Multiple central-place territories in wild young-of-the-year Atlantic salmon *Salmo salar*. J. Anim. Ecol. **77**(3): 448–457. doi:10.1111/j.1365-2656.2008.01360.x. PMID:18248384.

Steingrímsson, S.Ó., and Grant, J.W.A. 2011. Determinants of multiple central-place territory use in wild young-of-the-year Atlantic salmon *Salmo salar*. Behav. Ecol. Sociobiol. **65**(2): 275–286. doi:10.1007/s00265-010-1042-9.

Tunney, T.D. 2008. Foraging mode of wild salmonids in Icelandic streams. M.Sc. thesis, Department of Biology, University of Iceland, Reykjavík, Iceland.

Valdimarsson, S.K., and Metcalfe, N.B. 2001. Is the level of aggression and dispersion in territorial fish dependent on light intensity? Anim. Behav. **61**(6): 1143–1149. doi:10.1006/anbe.2001.1710.

Venter, O., Grant, J.W.A., Noel, M.V., and Kim, J.W. 2008. Mechanisms underlying the increase in young-of-the-year Atlantic salmon (*Salmo salar*) density with habitat complexity. Can. J. Fish. Aquat. Sci. **65**(9): 1956–1964. doi:10.1139/F08-106.

Verwaijen, D., and Van Damme, R. 2008. Wide home ranges for widely foraging lizards. Zoology, **111**(1): 37–47. doi:10.1016/j.zool.2007.04.001. PMID:17997294.

Risks of ignoring fish population spatial structure in fisheries management

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Abstract: Ignorance of spatial structures in fisheries management may lead to unexpected risks of overexploitation. Based on the information about small yellow croaker (*Larimichthys polyactis*) off the coast of China, we simulated a fish population consisting of three subpopulations mixing at intermediate levels, which was considered in the "true" spatial structure of the population in this study. Three scenarios of population spatial structure were assumed in assessing and managing this simulated fishery: (*i*) metapopulation, which has the same structure as the "true" population; (*ii*) three independent subpopulations, which overlook the exchanges among the subpopulations; and (*iii*) unit population, which completely ignores the population spatial structure. Corresponding approaches were applied to assess and manage each of these assumed fish populations. The management time period was assumed to be 10 years with two harvesting levels (i.e., maximum sustainable yield (MSY) and $f_{0.1}$). Assessing and managing the metapopulation as several independent populations could lead to a high probability of overexploitation. Managing the metapopulation as a unit population could lead to local depletion. Use of MSY as a management target may be risk prone in the existence of a metapopulation, and use of a fishing mortality lower than $f_{0.1}$ as a management target is more desirable.

Résumé : Ne pas tenir compte des structures spatiales dans la gestion de la pêche peut mener à des risques imprévus de surexploitation. En utilisant des données sur la courbine jaune (*Larimichthys polyactis*) du large de la côte de Chine, nous avons simulé une population de poissons comprenant trois sous-populations qui se mélangent à des niveaux intermédiaires, ce qui est considéré la « véritable » structure spatiale de la population dans notre étude. Nous avons supposé trois scénarios de structure spatiale de la population dans l'évaluation et la gestion de cette pêche simulée, (*i*) une métapopulation qui a la même structure que la population « véritable », (*ii*) trois sous-populations indépendantes, ce qui néglige les échanges entre les sous-populations et (*iii*) une population unitaire qui ignore complètement la structure spatiale de la population. Nous avons utilisé des méthodes semblables pour évaluer et gérer chacune de ces populations hypothétiques de poissons. Nous avons déterminé une période de gestion de 10 ans avec deux niveaux de récolte (c.-à-d. le rendement maximum durable (MSY) et $f_{0.1}$). L'évaluation et la gestion de la métapopulation comme plusieurs populations indépendantes pourraient mener à une forte probabilité de surexploitation. La gestion de la métapopulation comme une population unitaire pourrait provoquer des déplétions locales. L'utilisation de MSY comme cible de gestion peut entraîner des risques, s'il existe une métapopulation; l'utilisation d'une mortalité due à la pêche inférieure à $f_{0.1}$ comme cible de gestion semble donc plus désirable.

[Traduit par la Rédaction]

Introduction

A metapopulation is defined as a group of subpopulations in which local subpopulation dynamics are driven in part by dispersal from other subpopulations in the group (Hanski 1999). Many studies suggest that metapopulations are common and their dynamics are strongly influenced by spatial attributes of habitat patches (Lindenmayer et al. 2001; Schtickzelle et al. 2005), life history patterns, and distances and dispersal rates among local subpopulations (Sawchik et al. 2002; Curtis and Naujokaitis-Lewis 2008).

In marine fisheries, two important factors should be considered when we evaluate impacts of exploitation on fish population dynamics: (*i*) population spatial structure resulting from dispersal and movement of fish in different life history stages (Dieckmann at al. 1999) and (*ii*) spatial dynamics of fishing efforts often resulting from spatial variability in population density (Botsford et al. 2009).

In most marine species, early life history is characterized by a planktonic larval stage. Because of the mainly passive nature of the larval stage, they are often carried over large distances by the dominant ocean currents (Shanks et al. 2003; Kinlan et al. 2005; Trakhtenbrot et al. 2005). When the adult stage is predominantly sessile, larval dispersal is

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the main cause of spatial structure in marine populations. However, for many species the adult stage is actually more mobile than the larval stage. In such cases, this provides additional connectivity over a broad range of spatial scales, ranging from daily movement over metres to migrations over thousands of kilometres.

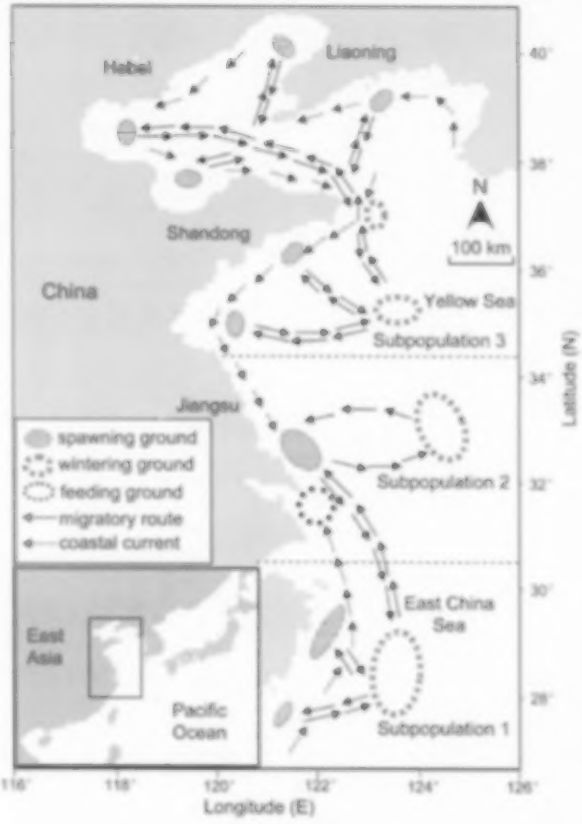
Many studies suggest that increasingly more marine fish populations should be considered as metapopulations, which are spatially structured and divided into several subpopulations mixing in intermediate levels (Kritzer and Sale 2004). This implies that it is necessary to control the repartition of the fleets on different fishing zones based on known spatial structures and interactions among subpopulations in fisheries management (Mchich et al. 2006). However, in many cases spatial structures are often ignored, and fish populations are considered as single and well-mixed populations or identical and independent populations in assessment and management (Botsford et al. 2009), often leading to over-exploitation (Stephenson 1999). The importance of considering spatial structure in the management of metapopulation fisheries was described in several studies (e.g., Hutchings 2000; Hutchinson 2008). Heifetz and Quinn (1998) showed that it is critical to consider movement and spatial structures in management. Hart and Cadrin (2004) developed a metapopulation approach to assess the yellowtail flounder (*Limanda ferruginea*) fishery. Heath et al. (2008) developed a metapopulation model in the case of North Sea cod (*Gadus morhua*) and showed the importance of considering population spatial structure in the management. More studies are still needed to improve our understanding of impacts of population spatial structure on fisheries stock assessment and management.

Based on the information about the small yellow croaker, *Larimichthys polyactis* in China, we simulated a metapopulation consisting of three subpopulations with migrations among the subpopulations. We then evaluated consequences of ignoring fish population spatial structure in metapopulation stock assessment and management.

Materials and methods

The small yellow croaker is a bentho-pelagic fish species of family Sciaenidae, inhabiting coastal waters and estuaries (Seikai National Fisheries Research Institute 2001). It supports important fisheries in Korea, Japan, and China. The species is widely distributed in the Bohai Bay and the Yellow and East China seas of the Northwest Pacific Ocean (Seikai National Fisheries Research Institute 2001). The small yellow croaker spawn pelagic eggs in spring, and its breeding grounds are located in the coastal area (Seikai National Fisheries Research Institute 2001). A long pelagic larval phase is observed in the small yellow croaker, which implies the high dispersal ability of this species. However, the small yellow croaker migrates back and forth regularly, between their spawning ground (also their feeding ground) in shallower water and three overwintering grounds (Fig. 1; Liu 1962). Based on the studies of its spawning migration patterns and morphological variables, Liu (1962) and Ikeda (1964) suggested that three groups existed throughout the range of small yellow croaker (Fig. 1). Molecular biology studies suggested weak but significant population genetic structures among the three subpopulations (Han et al. 2009; Lin et al. 2009). Weak

Fig. 1. China Coastal Current and migration routes and three subpopulations of small yellow croaker. The two horizontal dashed line divides the small yellow croaker into three subpopulations, based on the migration routes.



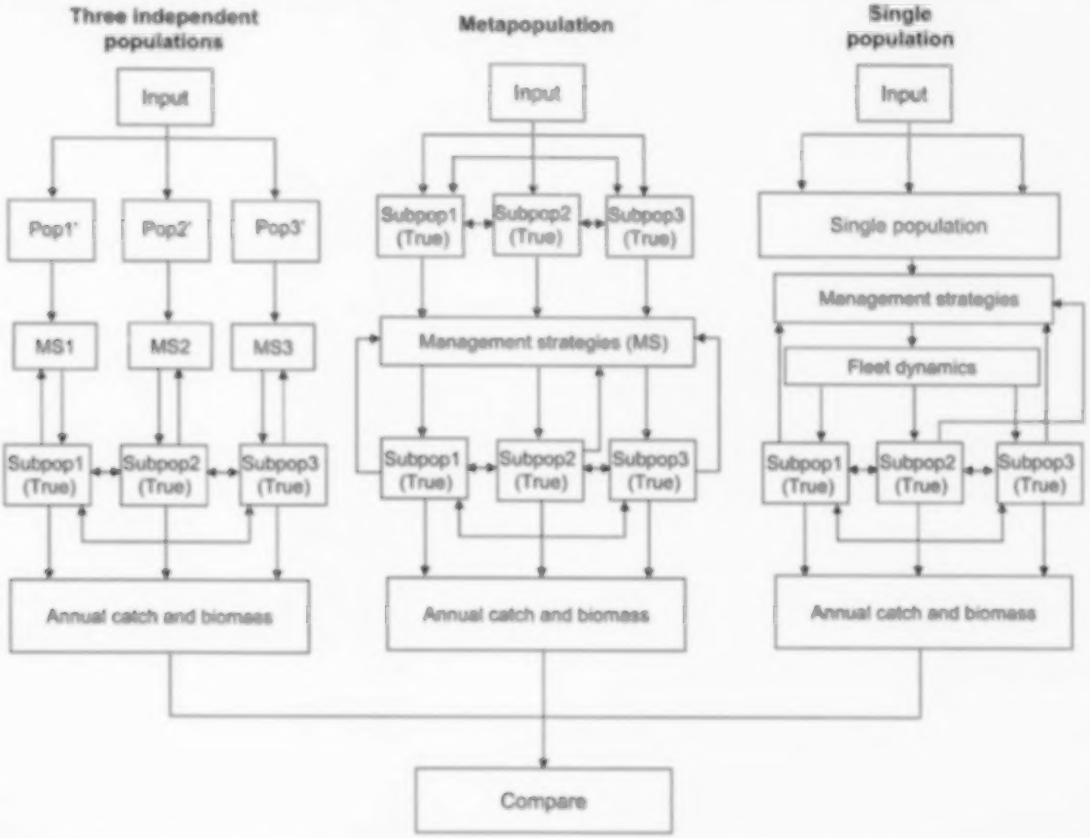
genetic structures indicated the existence of gene flow but limited individual exchanges among the subpopulations. The early life history, migration routes, population spatial structure, and intermediate levels of individual exchanges among the subpopulations imply that the small yellow croaker population is a metapopulation. Thus, this species is a good candidate to evaluate the impacts of spatial structure on stock assessment and management.

For this study, it was assumed that the metapopulation of the small yellow croaker in China consisted of three subpopulations. The subpopulation in the East China Sea (subpopulation 1) was distributed in the area to the south of 30.5°N. The South Yellow Sea subpopulation (subpopulation 2) was located in the area between 30.5°N and 34.5°N. The North Yellow Sea and Gulf of Bohai subpopulation (subpopulation 3) was located in the area to the north of 34.5°N (Fig. 1). We collected the yield (Chinese Fishery Yearbook Publishing House 2000–2009) and scientific survey catch-per-unit-effort (CPUE) data (Lin et al. 2006; Lin 2009; Chinese Fishery Yearbook Publishing House 2000–2009) for the small yellow croaker of the three subpopulations from 1999 to 2008. Owing to lack of reliable estimates, a constant migration rate was assumed based on the coastal current direction and speed in spring (Table 1; Li et al.

Table 1. Exchange rates among different subpopulations assumed based on the coastal current orientation and velocity in spring seasons.

	From		
To	Subpopulation 1	Subpopulation 2	Subpopulation 3
Subpopulation 1	—	0.15	0.05
Subpopulation 2	0.18	—	0.15
Subpopulation 3	0.08	0.1	—

Fig. 2. The design of this study. Three spatial options were considered in the management: (*i*) metapopulation structure assuming that the spatial structure is correctly identified; (*ii*) three independent subpopulations ignoring the exchange among subpopulations; (*iii*) unit population ignoring the spatial structure. The parameters were estimated based on different spatial options and then used to estimate the harvest level. The harvest level was applied to the metapopulation (true population structure) to manage the fishery based on assumed spatial structures to generate annual yield and stock biomass data.



2000), because larval dispersal of this species with the China Coastal Current was considered to play a primary role in the formulation of their spatial structure (Han et al. 2009; Lin et al. 2009).

We simulated a metapopulation with three subpopulations and limited movements between the subpopulations. Although the fishery was simulated based on the information available on the small yellow croaker population, it should not be considered as exactly the same as the small yellow croaker fishery, because many key parameters had to be assumed. Nevertheless, such a simulated fishery with known spatial structure is sufficient for this study.

The "true" population structure assumed for the simulations was that of a metapopulation. Three spatial options were considered in the assessment and management: (*i*) metapopulation structure, which implies we "correctly" delineated the "true" spatial structure of the population; (*ii*) three independent subpopulations with no intersubpopulation mixing, which implies that we considered the separation of the three subpopulations, but ignored the migration among the three subpopulations; and (*iii*) unit population, which means we ignored the spatial structure existing in the "true" population. These three spatial options correspond to "correct", "partially correct", and "wrong" interpretation of the population spatial structure, respectively, in the assessment and management.

Four steps were taken in evaluating the risk of ignorance

of the spatial structures in fisheries management (Fig. 2). First, an operating model was developed to define the "true" dynamics and spatial structure of a target metapopulation. The operating model was fitted to the data of Chinese small yellow croaker fishery to estimate parameters for simulating "correctly" defined metapopulation dynamics. Second, random variability was added to the data generated from the first step to generate simulated "observed" data for the metapopulation. The population parameters were estimated for the respective three spatial options considered in this study. Corresponding management harvest control rules were developed. Third, these harvest rules were applied to manage the simulated fishery to generate a set of annual yield and stock biomass data. Finally, we used yield, population biomass, and stock status to compare managements based on different population spatial structures assumed in the assessment.

In summary, we simulated a fishery with a defined spatial structure as a "true" fishery and tried to manage this fishery under the scenarios having perfect, partial, and wrong knowledge about the population structure. We compared the performance of management under these three scenarios to determine the importance of population spatial structure in fisheries assessment and management.

Operating model

The "true" population dynamics were simulated using a surplus production model with the consideration of migrations among the three subpopulations. The metapopulation structure likely results from larval transportation in their pelagic phase and adults in each subpopulation having migration routes that maintain isolation among subpopulations and result in limited mixing among adults of differing "home" populations. However, it is impossible to know the relative contributions of larval transportation and adult movements to the exchange of fish among the subpopulations. Thus, the ranges of exchange rates among subpopulations were assigned somewhat arbitrarily based on relative differences in coastal currents (Li et al. 2000). For lack of information, the exchange rates were assumed to be density-independent.

The population dynamics of the metapopulation were given by the equation

$$(1)\quad B_{i,t+1} = B_{i,t} + r_i B_{i,t}\left(1 - \frac{B_{i,t}}{K_i}\right) - \sum_{j \neq i}^{R} \theta_{i\to j} B_{i,t} + \sum_{j \neq i}^{R} \theta_{j\to i} B_{j,t} - C_{i,t}$$

where $B_{i,t}$ is the biomass of subpopulation i in year t; r_i is the growth rate of subpopulation i; K_i is the carrying capacity of subpopulation i; $\theta_{i\to j}$ is the annual rate of movement from subpopulation i to j, describing the proportion of individuals moving out of subpopulation i to subpopulation j in a year; and $C_{i,t}$ is the catch of subpopulation i in year t.

The annual predicted CPUE of subpopulation i in year t, $\hat{U}_{i,t}$, is calculated as

$$(2)\quad \hat{U}_{i,t} = q_i B_{i,t} e^{\varepsilon} \qquad \varepsilon \sim N(0, \sigma^2)$$

where q_i is the catchability of subpopulation i; ε is the normally distributed errors; σ is the standard deviation of ε; and $B_{i,t}$ is defined as above. We estimated the parameters of the metapopulation (r, K, q, and B_0) by minimizing the following objective function:

$$(3)\quad \mathrm{RSS} = \sum_{i}^{R}\sum_{t}^{T} (\log \hat{U}_{i,t} - \log U_{i,t})^2$$

where $\hat{U}_{i,t}$ is the predicted CPUE of subpopulation i in year t; $U_{i,t}$ is the observed CPUE of subpopulation i in year t; R is the total number of subpopulations; and T is the total number of years for which data were available.

Estimation model

For the metapopulation management option, we used the true parameters estimated using eqs. 1 to 3 to develop harvest control rules. For the three independent subpopulations management option, we estimated the parameters separately for each subpopulation as if they were the independent populations. The dynamics of each population can be described as

$$(4)\quad B_{i,t+1} = B_{i,t} + r_i B_{i,t}\left(1 - \frac{B_{i,t}}{K_i}\right) - C_{i,t}$$

where K_i is the carrying capacity of "population" i; and $B_{i,t}$, r_i, and $C_{i,t}$ are defined as above. Parameters were then estimated using eqs. 4, 2, and 3 for each subpopulation separately.

For a unit population management option, we used the following equation to describe the population dynamics:

$$(5)\quad B_{\mathrm{all},t+1} = B_{\mathrm{all},t} + r_{\mathrm{all}} B_{\mathrm{all},t}\left(1 - \frac{B_{\mathrm{all},t}}{K}\right) - C_{\mathrm{all},t}$$

where $B_{\mathrm{all},t}$ is the "current population biomass" for all the three subpopulations treated as if they were a unit population in year t. The population parameters were estimated using eqs. 5, 2, and 3.

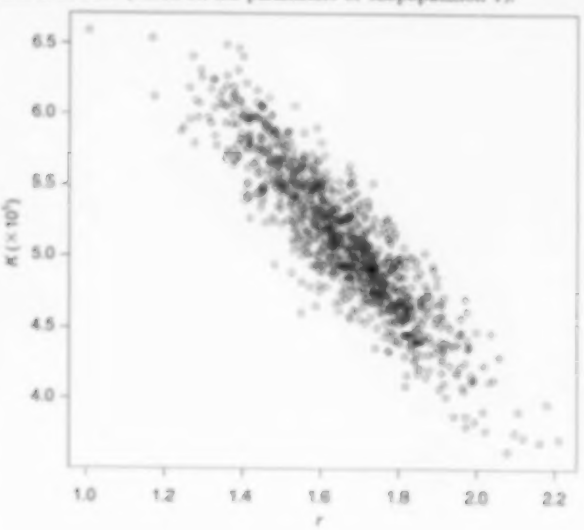
Uncertainty can be incorporated in a surplus production model in different ways. One approach is to simulate parameters r and K from a given multivariate normal distribution defined by a vector of means and variance–covariance matrix of the parameters (see Bard 1974, p. 316 for the algorithm). Many studies suggest a negative correlation between r and K (Bell 1990; Underwood 2007). We developed a variance–covariance matrix of r and K, with a correlation coefficient of –0.9 and the coefficient of variation (CV) of 10%. This is equivalent to the level of the correlation coefficient and CV in estimating r and K in the parameter estimation of this study when we fitted the production model to CPUE and catch data for the small yellow croaker. The variance–covariance matrix $\boldsymbol{\Sigma}$ was defined as

$$(6)\quad \boldsymbol{\Sigma} = \begin{pmatrix} \sigma_r^2 & \rho\sigma_r\sigma_K \\ \rho\sigma_r\sigma_K & \sigma_K^2 \end{pmatrix}$$

where σ_r is standard deviation of parameter r; σ_K is standard deviation of parameter K; and ρ is the correlation coefficient. One thousand pairs of normally distributed r and K values were then simulated using the variance–covariance matrix (Fig. 3) as

$$(7)\quad \begin{pmatrix} r_s \\ K_s \end{pmatrix} \sim N\left[\begin{pmatrix} r \\ K \end{pmatrix}, \boldsymbol{\Sigma}\right]$$

Fig. 3. The plot of 1000 pairs of r and K simulated from a multivariate normal distribution with correlation coefficient between r and K of –0.9 (based on the parameters of subpopulation 1).



where r_s and K_s are simulated r and K. This is similar to the approach used by Chen (1997).

Harvest control rule

We developed the following harvest control rule to determine annual catch using the parameters estimated for the three different spatial structure options in the management. We set the biomass limit to be $0.25B_{MSY}$ and the management target to be B_{MSY}, where MSY is maximum sustainable yield. When the current biomass was higher than the management target, we used the calculated annual catch. If the current biomass was less than the target biomass but more than the biomass limit, we determined the next year's catch level as follows:

(8) (current biomass – biomass limit)
× (catch target level)/(biomass target – biomass limit)

If the current biomass was less than the biomass limit, the fishery was closed with no catch for the next year.

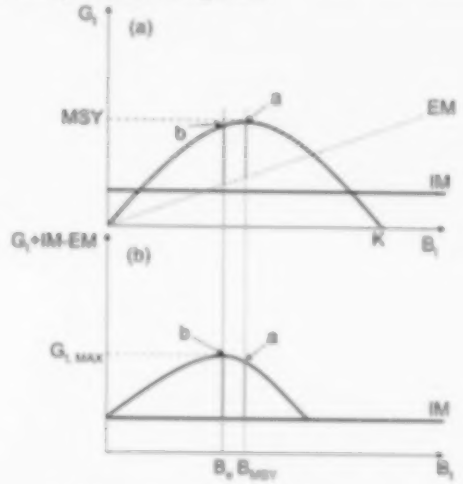
For the metapopulation management option, we had to consider the movement among subpopulations. Thus, we calculated a Y_i to describe the biomass used to decide the harvest level. Y_i is the biomass at the beginning of the year plus the net immigration:

$$(9)\qquad Y_{i,t} = B_{i,t} + \sum_{j\neq i}\theta_{j\to i}B_j - \sum_{i\neq j}\theta_{i\to j}B_i$$

The annual catch was then determined based on the harvest control rule defined above. For the other two spatial structure options, we used the biomass at the beginning of each year to determine the annual catch level based on the defined harvest control rule.

Many biological reference points can be used to determine total allowable catch (TAC) and catch level (Hilborn and Walters 1992; Quinn and Deriso 1999; Chen and Montgomery 1999). In this study, we used MSY and $f_{0.1}$ to determine TAC (Chen and Montgomery 1999; Quinn and Deriso 1999). For the three independent and unit population options, we used both to set target catch and compare their performance. In this case, MSY was calculated as $0.25r_iK_i$ (Hilborn and Walters 1992). The $f_{0.1}$ was defined by Chen and Montgomery (1999) as

Fig. 4. The two levels of total allowable catch used in the metapopulation management. The equilibrium catch level at B_{MSY} is the point "*a*". The catch level at maximum growth (equilibrium point at slope equal to 0) of a subpopulation is point "*b*". G_i (panel *a*) is the growth of the subpopulation in 1 year when the biomass of the subpopulation in the beginning of the year equals it in the end of the year. B_i is the biomass at the beginning of the year. The line labeled "EM" is the emigration of target subpopulation. The line labeled "IM" is the immigration from other subpopulations to the target subpopulation. G_i + IM – EM (panel *b*) is used to design the catch level for metapopulation management.



$$(10)\qquad \left.\frac{\partial C(E)}{\partial E}\right|_{E=f_{0.1}/q} = 0.1\left.\frac{\partial C(E)}{\partial E}\right|_{E=0}$$

where $C(E)$ is the equilibrium yield corresponding to effort E (Punt 1993). From the above equation and eq. 4, $f_{0.1}$ can be calculated as $f_{0.1} = 0.45r$ (Chen and Montgomery (1999).

However, determining TAC for the metapopulation option was more complicated because it needs to consider subpopulation mixing. The MSY of non-metapopulation management is the equilibrium yield at B_{MSY} ($B_{MSY} = 0.5K_i$; Hilborn and Walters 1992). The yield at B_{MSY} is defined as the catch level at biomass supporting the highest population growth (Hilborn and Walters 1992) and leading to the next year's biomass equal to the last year's biomass. However, the equilibrium yield at B_{MSY} in the metapopulation option is not the same as the maximum equilibrium yield (Fig. 4). This can be described as

$$(11)\qquad C_{i,t} = r_iB_{i,t}\left(1-\frac{B_{i,t}}{K_i}\right) - \sum_{j\neq i}^{n}\theta_{i\to j}B_{i,t} + \sum_{j\neq i}^{n}\theta_{j\to i}B_{j,t}$$

When B_{MSY} of $0.5K_i$ is input into the above equation, the yield at B_{MSY} becomes

Table 2. Simulation scenarios considered in this study.

Scenario	Management option	Total allowable catch	Uncertainty	CV (%)	Exchange rate
1	Metapopulation	Point at B_{MSY}	Without	10	1×
2	Metapopulation	Equilibrium point at slope equal to 0	Without	10	1×
3	Metapopulation	Point at B_{MSY}	With	10	1×
4	Metapopulation	Equilibrium point at slope equal to 0	With	10	1×
5	Three populations	MSY	With	10	1×
6	Unit population	MSY	With	10	1×
7	Metapopulation	Point at B_{MSY}	With	5	1×
8	Three populations	MSY	With	5	1×
9	Unit population	MSY	With	5	1×
10	Metapopulation	Point at B_{MSY}	With	20	1×
11	Three populations	MSY	With	20	1×
12	Unit population	MSY	With	20	1×
13	Metapopulation	Point at B_{MSY}	With	10	0.5×
14	Three populations	MSY	With	10	0.5×
15	Unit population	MSY	With	10	0.5×
16	Metapopulation	Point at B_{MSY}	With	10	1.5×
17	Three populations	MSY	With	10	1.5×
18	Unit population	MSY	With	10	1.5×
19	Metapopulation	90% of fishing effort at B_{MSY}	With	10	1×
20	Three populations	$f_{0.1}$	With	10	1×
21	Unit population	$f_{0.1}$	With	10	1×
22	Metapopulation	0.5 × point at B_{MSY}	With	10	1×
23	Three populations	0.5 × MSY	With	10	1×
24	Unit population	0.5 × MSY	With	10	1×
25	Metapopulation	1.5 × point at B_{MSY}	With	10	1×
26	Three populations	1.5 × MSY	With	10	1×
27	Unit population	1.5 × MSY	With	10	1×

Note: B_{MSY} is the management target for annual biomass catch, where MSY is maximum sustainable yield (see Fig. 4); CV is coefficient of variation. Exchange rate is compared with the base case rate defined in Table 1.

$$(12)\qquad C_{i,t} = \frac{r_i K_i}{4} - \frac{K_i}{2}\sum_{i\neq j}\theta_{i\to j} + \sum_{j\neq i}\theta_{j\to i}B_{j,t}$$

The maximum equilibrium yield (Fig. 4) can be defined as

$$(13)\qquad \frac{\partial C_{e,t}}{\partial B_i} = \left(r_i - \sum_{i\neq j}\theta_{i\to j}\right) - \frac{2B_i r_i}{K_i} = 0$$

where $C_{e,t}$ is the yield leading to the next year's biomass equal to the last year's biomass, which is the same as eq. 11.

From eqs. 8 and 10, the equilibrium can be determined as

$$(14)\qquad C_{i,t} = \frac{K_i\left(r_i - \sum_{i\neq j}\theta_{i\to j}\right)^2}{4r_i} + \sum_{j\neq i}\theta_{j\to i}B_{j,t}$$

The estimation of $f_{0.1}$ for the metapopulation option is also complicated and could not be simply determined as $0.45r$. Because the fishing mortality at MSY level is $0.5r$ (Quinn and Deriso 1999; Chen and Montgomery 1999), we used 90% of the harvest level at B_{MSY} as the catch level for the metapopulation option using $f_{0.1}$ as biological reference point (Chen and Montgomery 1999).

Simulation of the management

The harvest rules, derived above, were applied to manage the simulated fishery. For the metapopulation and three independent subpopulation options, the harvest level of each year and dynamics of subpopulations were estimated with the harvest control rules. For the unit population option, the fleet dynamics were also considered, with the allocation of fishing effort and TAC among the three subpopulations being determined based on the relative difference in CPUE among the three fishing areas targeting the three subpopulations. The proportion of TAC of subpopulation i in year t was determined as

$$(15)\qquad \text{TAC proportion of subpopulation } i = \frac{U_{i,t}}{\sum_{j=1}^{R} U_{j,t}}$$

where $U_{i,t}$ is the observed CPUE of subpopulation i in year t; and R is the total number of the subpopulations. This implies that fishermen are mobile and have knowledge about the performance of other fishermen and where the fish are located (Hilborn and Walters 1992; Wilson et al. 1999). We used 10 years as management time period in this study, which is long enough to address the objective of this study.

Simulation scenarios

To evaluate risks of ignoring fish population spatial structure, we developed various simulation scenarios in this study (Table 2). Scenarios 1, 2, 3, 4, 7, 10, 13, 16, 19, 22, and 25 were based on the assumption that the population was managed under the metapopulation option (i.e., assumed spatial

structure in the assessment is correct); Scenarios 5, 8, 11, 14, 17, 20, 23, and 26 assumed that the population was managed under the three independent subpopulations option (i.e., assumed spatial structure does not consider movement among the subpopulations); and Scenarios 6, 9, 12, 15, 18, 21, 24, and 27 assumed that the population was managed under the unit population option (i.e., no spatial structure was assumed in the assessment; Table 2).

Scenarios 1 to 6 were used to test the TAC-based management with catch being set at MSY. Different biological references in the simulation were assumed as the MSY in the metapopulation management. MSY was calculated based on B_{MSY} for Scenarios 1 and 3 (Fig. 4), and MSY was calculated at equilibrium point with the slope of 0 for Scenarios 2 and 4 (Fig. 4). For the other scenarios (i.e., 5 and 6), MSY was calculated based on B_{MSY}. Scenarios 1 and 2 were the control scenarios without consideration of uncertainty (Table 2).

Scenarios 7 to 12 were used to evaluate different levels of uncertainty. Scenarios 7 to 9 used 5% of CV and Scenarios 10 to 12 used 20% of CV for parameters r and K to simulate the uncertainty (Table 2).

Scenarios 13 to 18 were used to evaluate different levels of exchange rate. Scenarios 13 to 15 used 0.5 times the exchange rates for the base case described (Table 2), and Scenarios 16 to 18 used 1.5 times the exchange rates for the base case (Table 2).

Scenarios 19 to 27 were used to test different catch levels. Scenarios 19 to 21 used a relative robust fishing mortality level $f_{0.1}$ (Chen and Montgomery1999). Scenarios 22 to 24 used a low catch level (0.5 × MSY), and Scenarios 25 to 27 used a higher catch level (1.5 × MSY).

Results

The mean values of parameters for the three spatial structure options were summarized (Table 3). The B_0 and K values in subpopulations 1 and 3 and the growth rate r of subpopulation 2 were higher than those for the "true" population when the migration was not considered (Table 3). The value of growth rate r of each subpopulation estimated in this study was common in family Sciaenidae (http://www.fishbase.org/Nomenclature/NominalSpeciesList.cfm?family=Sciaenidae), suggesting that the simulated population is biologically reasonable.

Boxplots of stock biomass and annual catch are presented for each simulated scenario (Figs. 5–13). The box depicts the interquartile range; the horizontal line through the box represents the median score; and the whiskers represent 95% confidence intervals.

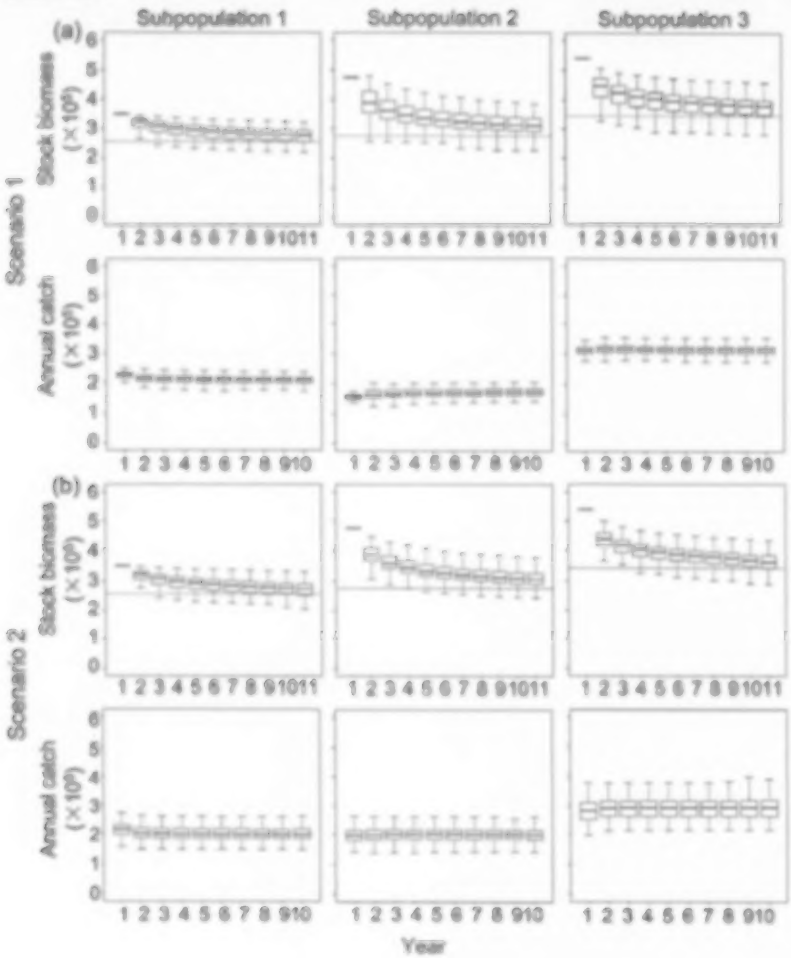
For the control cases without uncertainty (i.e., Scenarios 1 and 2; Table 2), all subpopulations had stable stock biomass over the simulation time period (Fig. 5). The annual biomass was kept above the mean value of the biomass management target, and the annual yields of each subpopulation were stable (Fig. 5). For Scenarios 3 and 4 considering the uncertainty of parameter estimates, over-exploitation of the subpopulations occurred in some of the simulation runs, indicating the possibility that over-exploitation existed (Fig. 6), although the annual yield was stable for each subpopulation. The median values of population biomass were higher than the management target for Scenario 3, but lower than the

Table 3. The average population parameters and biomass management targets estimated for the three spatial options considered in the management.

			r			K ($\times 10^5$)				Biomass targets ($\times 10^5$)		
Management strategy	Area	B_0 ($\times 10^5$)	$1 \times \theta$	$0.5 \times \theta$	$1.5 \times \theta$	$1 \times \theta$	$0.5 \times \theta$	$1.5 \times \theta$	q ($\times 10^{-4}$)	$1 \times \theta$	$0.5 \times \theta$	$1.5 \times \theta$
Metapopulation	Subpopulation 1	3.59	1.64	1.52	1.52	5.15	5.16	5.16	4.47	2.57	2.58	2.58
(true)	Subpopulation 2	4.61	1.24	1.61	1.39	5.57	5.43	5.49	6.54	2.78	2.72	2.75
	Subpopulation 3	5.11	1.8	1.62	1.99	6.99	6.92	7.03	6.03	3.49	3.46	3.51
Three	Population 1	3.75		1.41				5.16	4.53		2.58	
independent	Population 2	4.33		1.94			5.36		6.59		2.68	
populations	Population 3	5.22		1.8			6.99		5.73		3.5	
Unit population	Whole population	13.02		1.79			19.11		1.12		9.55	

Note: θ is the exchange rate shown in Table 1.

Fig. 5. Boxplots of stock biomass at the beginning of the years and annual catch of simulation Scenarios 1 (*a*) and 2 (*b*) defined in Table 2 (metapopulation management at MSY without uncertainties). The mean values of biomass management targets in the 1000 simulation runs are shown by horizontal dotted lines.



management target for subpopulation 2 in Scenario 4 (Fig. 6). For Scenario 5, which ignored the migration among subpopulations, over-exploitation was detected for all the three subpopulations (Fig. 6). The biomasses of subpopulations 2 and 3 were depleted, respectively, in the fourth and sixth years (Fig. 6). The yield of the two subpopulations continuously declined. Under a constant catch management regime, the biomass of subpopulation 1 declined substantially, leading to high probability of over-exploitation (Fig. 6). For Scenario 6, which ignored all of the spatial information of the metapopulation, the stock biomasses of all the three subpopulations were severely depleted early in the simulation and maintained very low levels of biomass (Fig. 6). The annual yields fluctuated greatly among years for all the subpopulations (Fig. 6).

For Scenarios 7, 8, and 9, 5% of CV was employed to simulate low uncertainty. The biomass under the metapopulation management option (Scenario 7; Fig. 7) remained stable and was close to the target. Under the independent population management option (Scenario 8; Fig. 7), the stock biomass of subpopulations 2 and 3 was stable at the level slightly lower than the management target. The stock biomass of all subpopulations under the unit population management option (Scenario 9; Fig. 7) was severely depleted early in the simulation. In contrast, for Scenarios 10, 11, and 12, 20% of CV was employed to simulate high uncertainty. Large variability could be seen in the simulation results, but the median catch and biomass values were similar to those for scenarios having 5% of CV, except the independent population management option, which maintained a stock biomass lower than that for the scenario with 5% of CV (Fig. 8).

For Scenarios 13 to 18, low and high exchange rates were employed. Half of exchange rate values shown in Table 2 were used in Scenarios 13, 14, and 15 (Fig. 9). Stock biomasses and catches under the single population management option were similar with the values under the metapopulation management scenarios, but severe depletion was still observed for the unit population management option.

In contrast, for Scenarios 16, 17, and 18, 1.5 times the exchange rate value shown in Table 3 were used. Biomasses under the single population management option (Scenario 17) were over-exploited to a level much lower than those for the metapopulation management option (Scenario 16). The subpopulations under the unit population management option (Scenario 18) declined moderately, though over-exploitation was observed (Fig. 10).

For Scenarios 20 and 21, which used $f_{0.1}$ instead of MSY to determine TAC, the annual stock biomass levels were higher and more stable than those for Scenarios 5 and 6 (Fig. 11). For Scenario 20, only light over-exploitation was detected, and both stock biomasses and yields were stable (Fig. 11). For Scenario 21 (Table 2), although a severe depletion happened in the beginning of the simulation, the stock biomass recovered and remained low but stable in the next several years (Fig. 11).

For Scenarios 22 to 27, different catch levels were assumed in management. For Scenarios 22, 23, and 24, TAC was set at 50% of MSY (Fig. 12). Stock biomasses were higher than the management target for all of the three scenarios (Fig. 12). For Scenarios 25, 26, and 27, TAC was set at 1.5 times of MSY (Fig. 13). Over-exploitation was detected for all of the three management scenarios. The stock biomasses were reduced, but maintained at a low level for Scenarios 25 and 26, but all the three subpopulations were close to extinction for Scenario 27, which ignored the population spatial structure (Fig. 13).

Discussion

Mixing among the subpopulations of a metapopulation, resulting from dispersal and migrations (Hansson 1991), has profound consequences to the persistence of populations, the distribution and abundance of species, and community structure (Dieckmann at el. 1999), and subsequently to fisheries assessment and management. Curtis and Naujokaitis-Lewis (2008) suggested that the dynamics of a metapopulation could be influenced by dispersal rates among populations. Failure to recognize complex population structure in management may lead to erosion of local subunits with unknown ecological consequences (Stephenson 1999). This study suggests that managing a metapopulation as several independent populations could lead to reduced stock biomass and high probability of over-exploitation. Ignorance of the exchange among the subpopulations might be responsible for the biases in estimating population parameters and subsequent determination of harvest level.

Age at sexual maturity becomes younger and the number of age groups is reduced as a result of over-exploitation for small yellow croakers (Lin 2009). The dominant age groups were 3–6 years old in 1963, 1–2 years in 1983, and 0–1 year in 2001; and the 0–1 age group consisted of 98.8% of the total population in 2001 (Lin 2009). From the 1990s, the spawning stock of small yellow croakers was mainly composed of the young of the year (Lin 2009). Recently, small yellow croaker was considered as a short-lived and fast-growing species. For such a stock with simple age structure, a production model is a good candidate for quantifying its population dynamics. Use of production model also does not require information such as recruitment, natural mortality, and selectivity, which is often not available. Thus we used surplus production model in this study.

This study shows that managing a metapopulation as a unit population could lead to local depletion. Botsford et al. (2009) suggested that the movement of fishermen had a significant effect on the dynamics of exploited, spatially structured marine populations. This study suggests that the depletion might be caused by the fishing fleet concentrating in areas of higher abundance.

For a metapopulation, two MSYs could be estimated: one estimated based on B_{MSY} and the other estimated by setting the slope of the equilibrium catch–effort curve equal to 0 (i.e., maximum rate). The catch level at B_{MSY} tended to be lower than the catch level at the maximum rate. Thus, the catch level at B_{MSY} tends to be a more conservative management reference point.

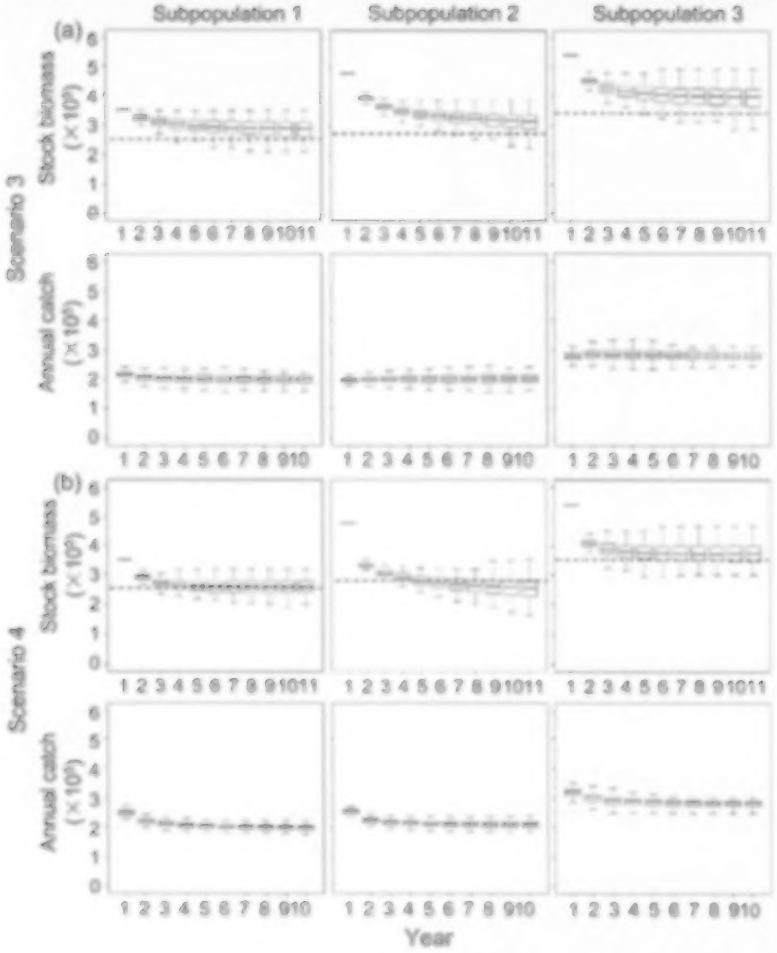
The quality of fisheries data has great impacts on the quality of stock assessment (Chen 2003). This study shows that uncertainties in estimating population parameters in metapopulation fisheries management can affect the management of a metapopulation. Over-exploitation of subpopulations occurred in some simulation runs for the scenarios with uncertainty, even when the spatial structure was correctly defined in the assessment. For the scenarios with lower levels of uncertainty, the variability was smaller and the stock biomass under the independent population option was stable at a higher biomass than that under the scenario with a higher uncertainty level. This suggests that reducing uncertainty associated with key population parameters such as K and r in stock assessment is important in metapopulation fisheries management.

The management of metapopulations is considered to be sensitive to the exchange rate (Curtis and Naujokaitis-Lewis 2008). In general, we expect that a high level of exchange among subpopulations makes the population similar to a unit population; but a low level of exchange makes the population similar to several independent populations. The results of this study are consistent with such an expectation. For the scenarios with low exchange rates, the independent population management option seemed to perform well; but for the scenarios with high exchange rates, the metapopulation managed under the unit population assumption performed better than it did with lower exchange rates. The sensitivity of the results to the exchange rates indicates the importance of knowing movement information for the assessment and management of a metapopulation.

We compared differences in management using $f_{0.1}$ and MSY-based reference points. We found that the annual stock biomass levels with $f_{0.1}$ as the management target were higher and more stable for the same spatial structure scenarios assumed in the management than those with the MSY as the management target. Therefore, exploitation based on MSY is risk-prone because the ability of the population to recover from exploitation might be impaired in the presence of uncertainties. Use of $f_{0.1}$ as management target is more desirable. The $f_{0.1}$ is often considered as a more conservative biological reference point (Chen and Montgomery 1999). Chen and Montgomery (1999) proposed to use $f_{0.1}$ as a biological reference point in a surplus production model and suggested it is more robust than the F_{MSY} regarding uncertainty.

For the scenarios with TAC being set at 1.5 times MSY, the high catch level led to high risks of over-exploitation in

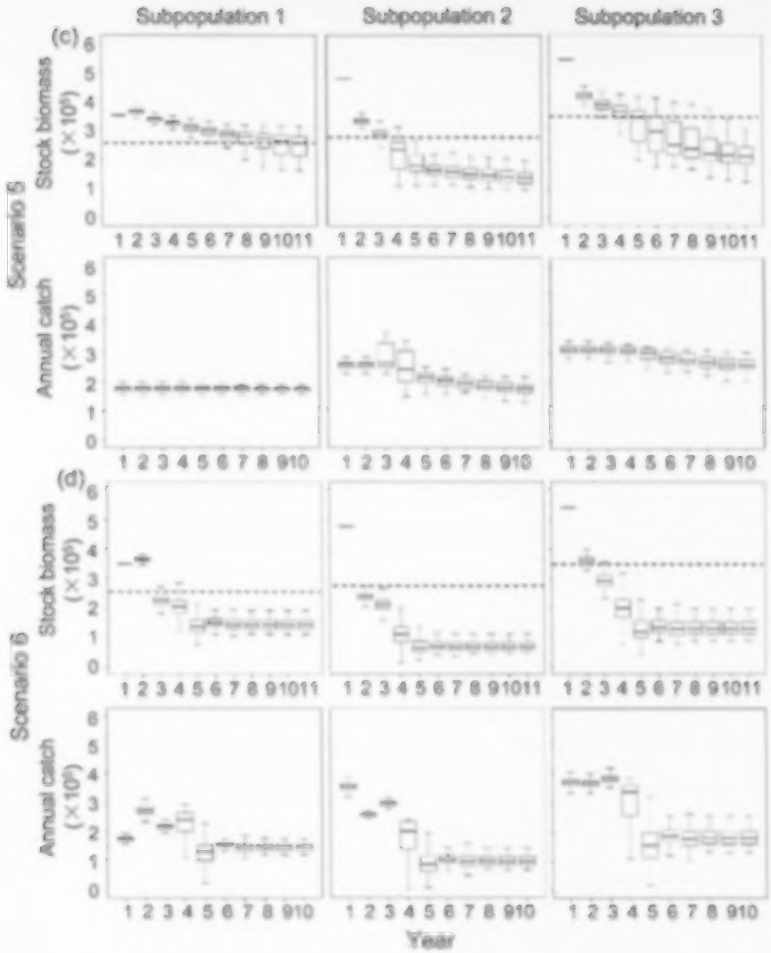
Fig. 6. Boxplots of stock biomass at the beginning of the years and annual catch for Scenarios 3 (*a*), 4 (*b*), 5 (*c*), and 6 (*d*) defined in Table 2 (managements with catch level at MSY for three spatial options). Scenario 3 (*a*) used the catch at B_{MSY} as MSY, and Scenario 4 (*b*) used the catch at equilibrium point at slope equal to 0. The biomass management targets of the "true" population are shown by horizontal dotted lines.



all the three management options. However, TAC of 50% MSY led to no over-exploitation being found in all management options, and stock biomass remained stable in the unit population option. Clearly reducing catch level can reduce the risks of over-exploitation in the management of metapopulations even though spatial structure of the population is wrongly assumed in the assessment and management.

Several studies suggest dangers of ignoring the spatial structure of fish populations in fisheries assessment and management (e.g., Hutchings 1996, 2000; Frank and Brickman 2000). Frank and Brickman (2000) developed a model based on the stock–recruitment (S–R) relationship with the Allee effect and suggested that biological reference points developed from the models ignoring multiple subpopulations and used in conventional fisheries assessment are likely to be inaccurate and possibly nonconservative. Hutchings (1996, 2000) shows the importance of subpopulation structure in the management of Atlantic cod (*Gadus morhua*) fisheries in Canada. Numerous studies suggested that ignorance of spatial structure in cod fisheries management might be responsible for the collapse of the cod populations (e.g., Hutchings 1996, 2000; Frank and Brickman 2000). Decrease in the Atlantic herring (*Clupea harengus*) population was also thought to be caused by failure to recognize the complex spatial structure (Stephenson 1999). The crustacean fisheries of Alaska (e.g., red king crab, *Paralithodes camtschaticus*) provide notable examples of fishery collapses (Wooster 1992), and ignorance of spatial structure in the management was considered one of the major reasons for the collapse (Orensanz et al. 1998). While the small yellow croaker is a short-lived and fast-growing species, the Atlantic cod and red king crab are considered long-lived species. Although different in their life history, the role of spatial structure and consequence of ignoring of population spatial structure in the management might be the same as long as they have metapopulation structure. Previous studies suggest the importance of spatial structure for long-lived species (e.g., Atlantic cod and red king crab), but limited work was done for short-lived species, which might be less likely to be affected by their spatial structure because of their high growth rates and small num-

Fig. 6. (*concluded*).



ber of age classes. Thus, this study complements the studies done previously for long-lived species.

Many short-lived species were considered to exhibit large variability in recruitment (e.g., Milton et al. 1996; Dichmont et al. 2006). As a short-lived species, the small yellow croaker also exhibit high variability in recruitment. Further, recent research observed an increasing trend in the recruitment rate of small yellow croaker (Lin 2009). However, in the simulation of this study, we did not explicitly incorporate temporal variability in recruitment because the surplus production model only considers intrinsic population growth, which combines recruitment, somatic growth, and natural mortality. The intrinsic growth, which includes recruitment, is dependent and varies with stock biomass. Thus, although we did not explicitly consider recruitment dynamics, we did implicitly consider temporal variability in recruitment.

The fishery was simulated based on the life history traits of small yellow croaker that spawn pelagic eggs in spring in the coastal area. However, recent studies showed that the spawning grounds of small yellow croaker had expanded offshore (Lin et al. 2008). Changing of spawning grounds might influence the migration routes and relative strength of three subpopulations. Its management implications thus need to be evaluated. The monitoring of the spatial distribution and movement of the population of small yellow croaker should be continued.

The assumptions used in this study are typical, and some values (e.g., exchange rates among the subpopulations) used were determined somewhat arbitrarily. The impacts of the assumptions and choices of the population parameters may influence the dynamics of fisheries population and should be evaluated more carefully. The harvest control rules considered in this study were limited. More harvest control rules and other management strategies such as marine reserves can be considered in management of metapopulations.

In summary, this study suggests that ignoring spatial structures in fisheries assessment and management may result in (*i*) biases in estimating population parameters; (*ii*) biases in estimating stock status; (*iii*) developing inappropriate management targets; (*iv*) recommending inappropriate harvest levels; and (*v*) depleting local populations. All of these might lead to higher risks of depleting fisheries populations. Thus,

Fig. 7. Boxplots of stock biomass at the beginning of years and annual catch of simulation Scenarios 7 (*a*), 8 (*b*), and 9 (*c*) defined in Table 2 (with 5% CV of uncertainties for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.

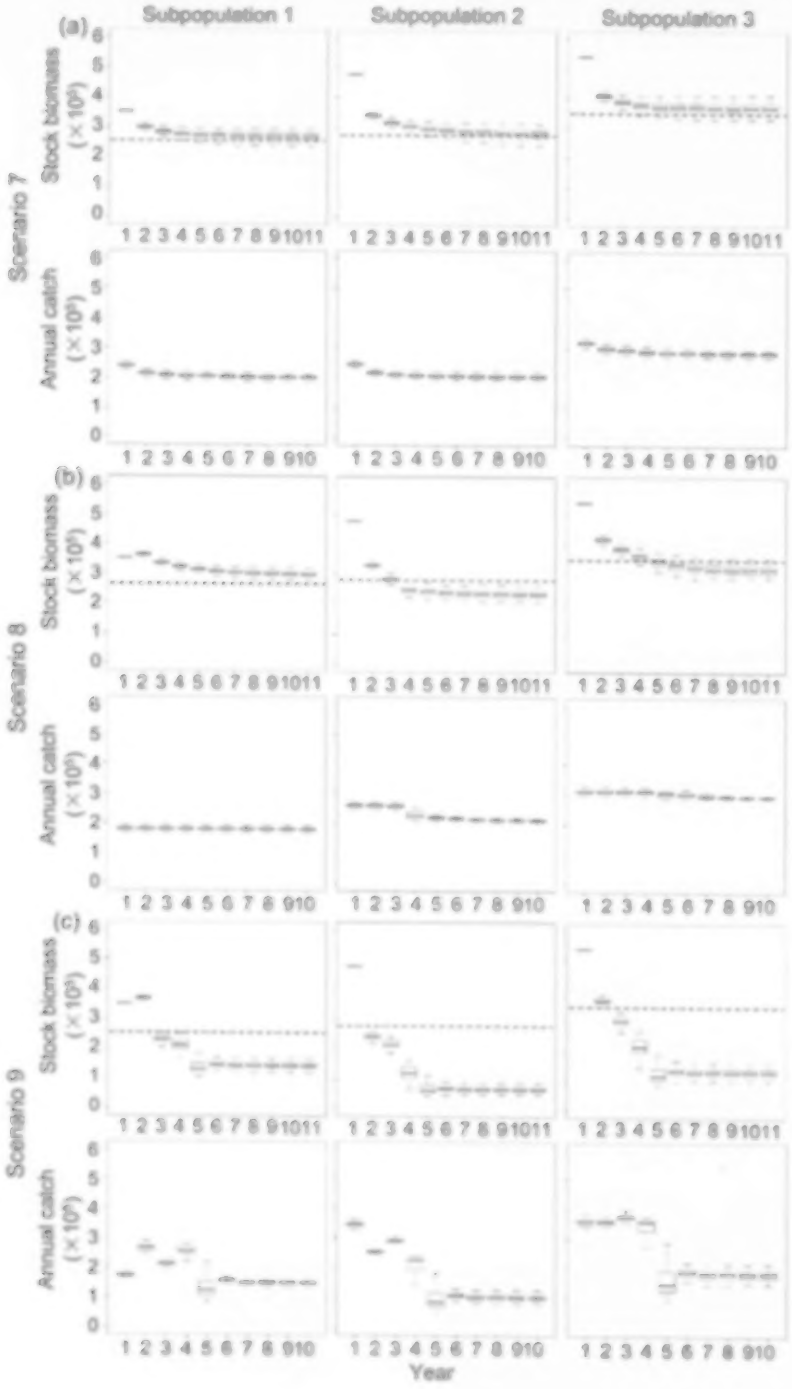


Fig. 8. Boxplots of stock biomass at the beginning of years and annual catch for Scenarios 10 (*a*), 11 (*b*), and 12 (*c*) defined in Table 2 (with 20% CV of uncertainties for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.

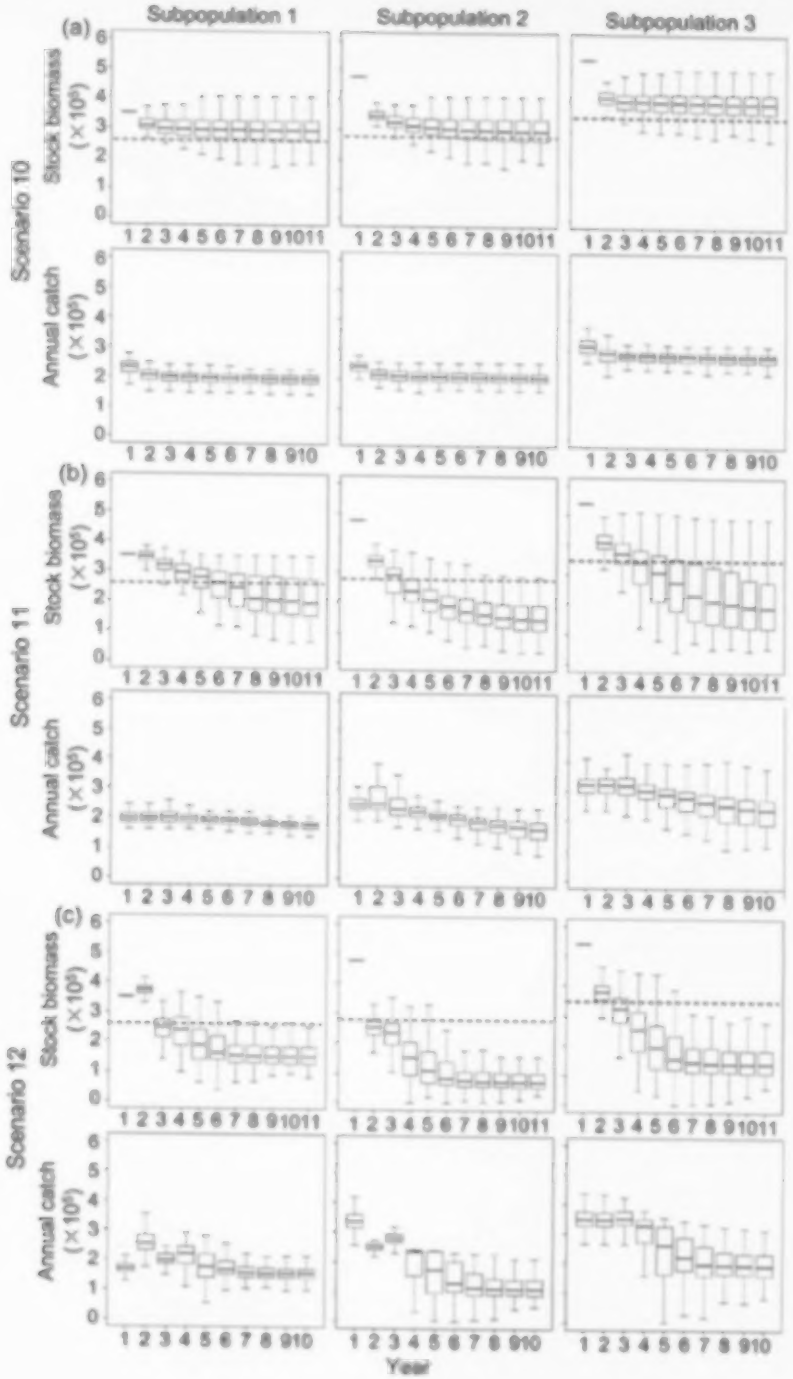


Fig. 9. Boxplots of annual biomass at the beginning of years and annual catch for Scenarios 13 (*a*), 14 (*b*), and 15 (*c*) defined in Table 2 (with 0.5 times the exchange rates for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.

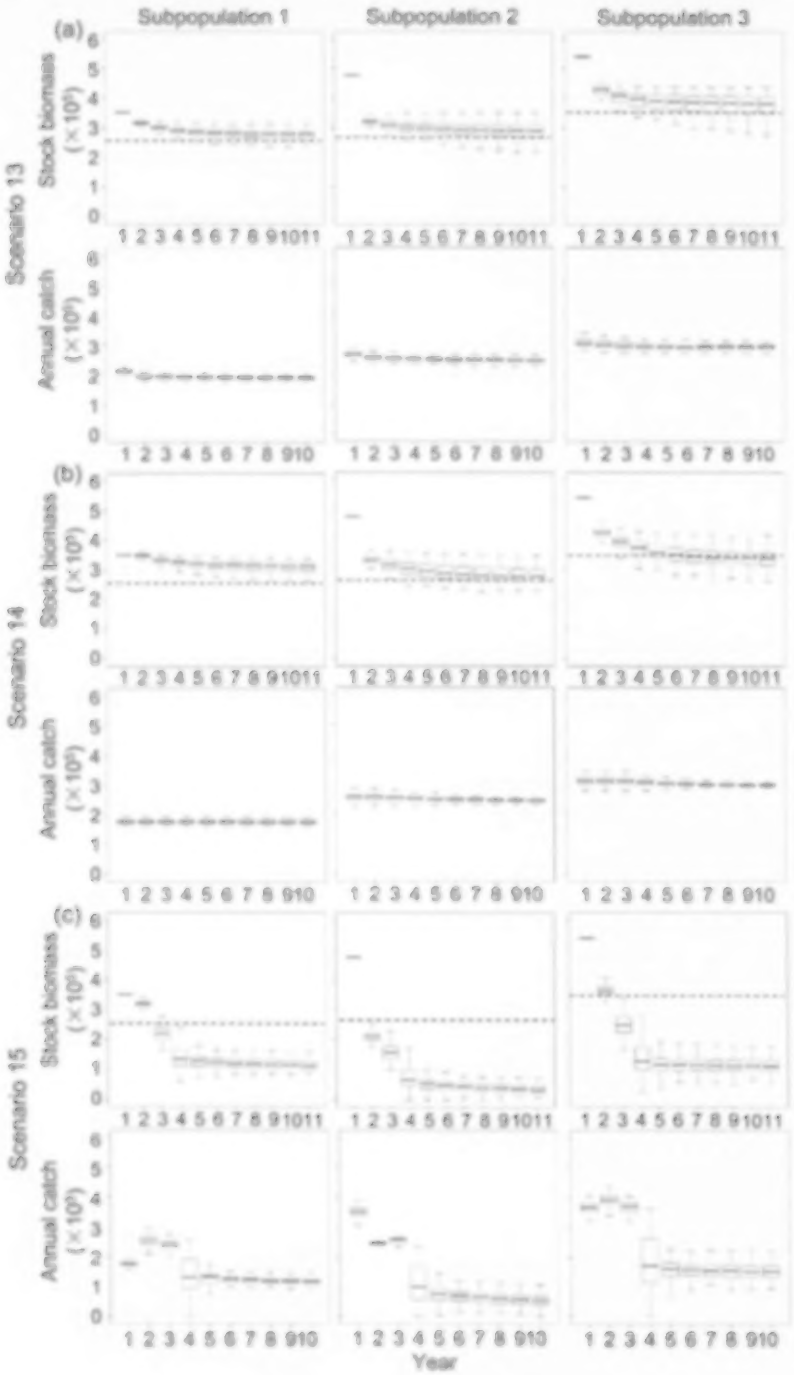


Fig. 10. Boxplots of stock biomass at the beginning of the years and annual catch for Scenarios 16 (*a*), 17 (*b*), and 18 (*c*) defined in Table 2 (with 1.5 times the exchange rates for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.

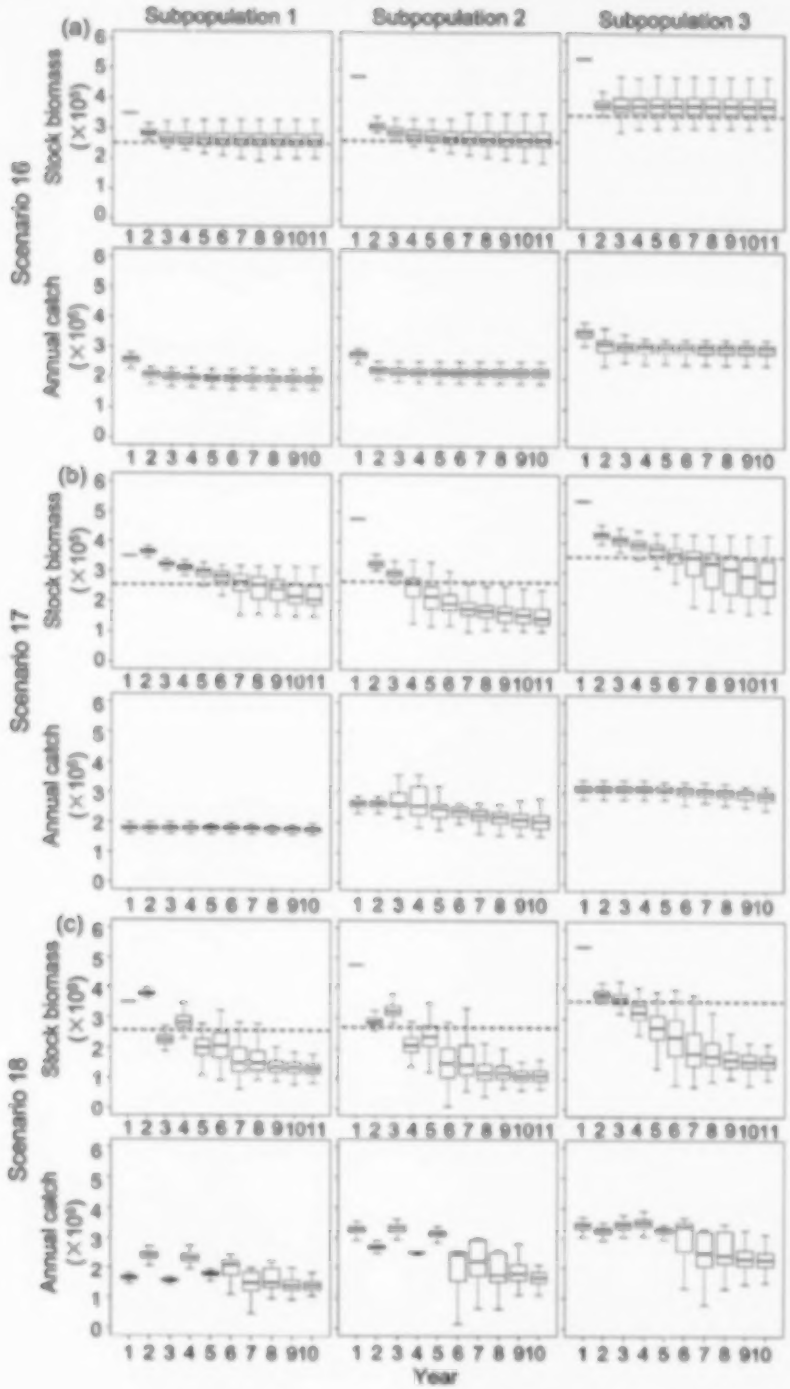


Fig. 11. Boxplot of stock biomass at the beginning of the years and annual catch for Scenarios 19 (*a*), 20 (*b*), and 21 (*c*) defined in Table 1 (scenarios under $f_{0.1}$ management for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.

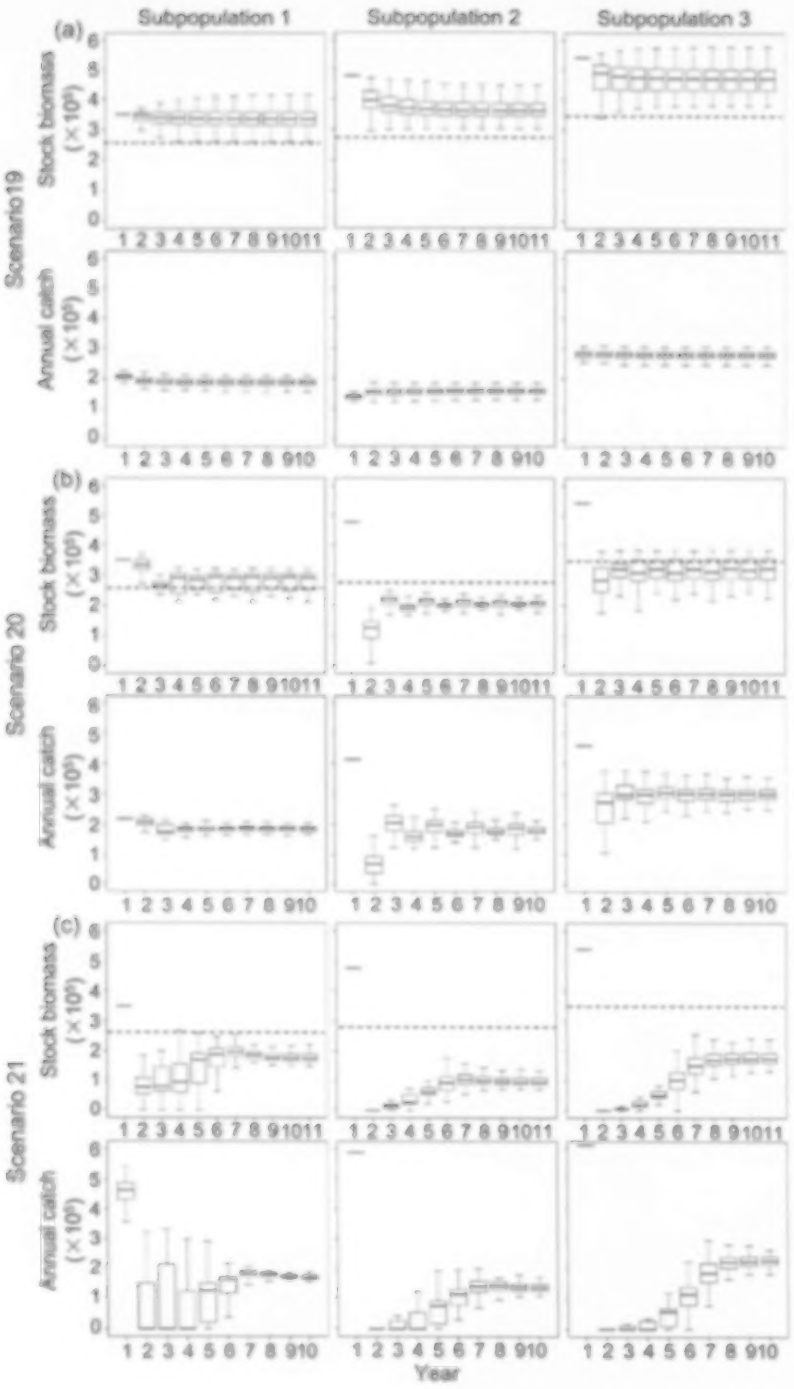


Fig. 12. Boxplots of stock biomass at the beginning of the years and annual catch for Scenarios 22 (*a*), 23 (*b*), and 24 (*c*) defined in Table 2 (scenarios with catch level at 0.5MSY for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.

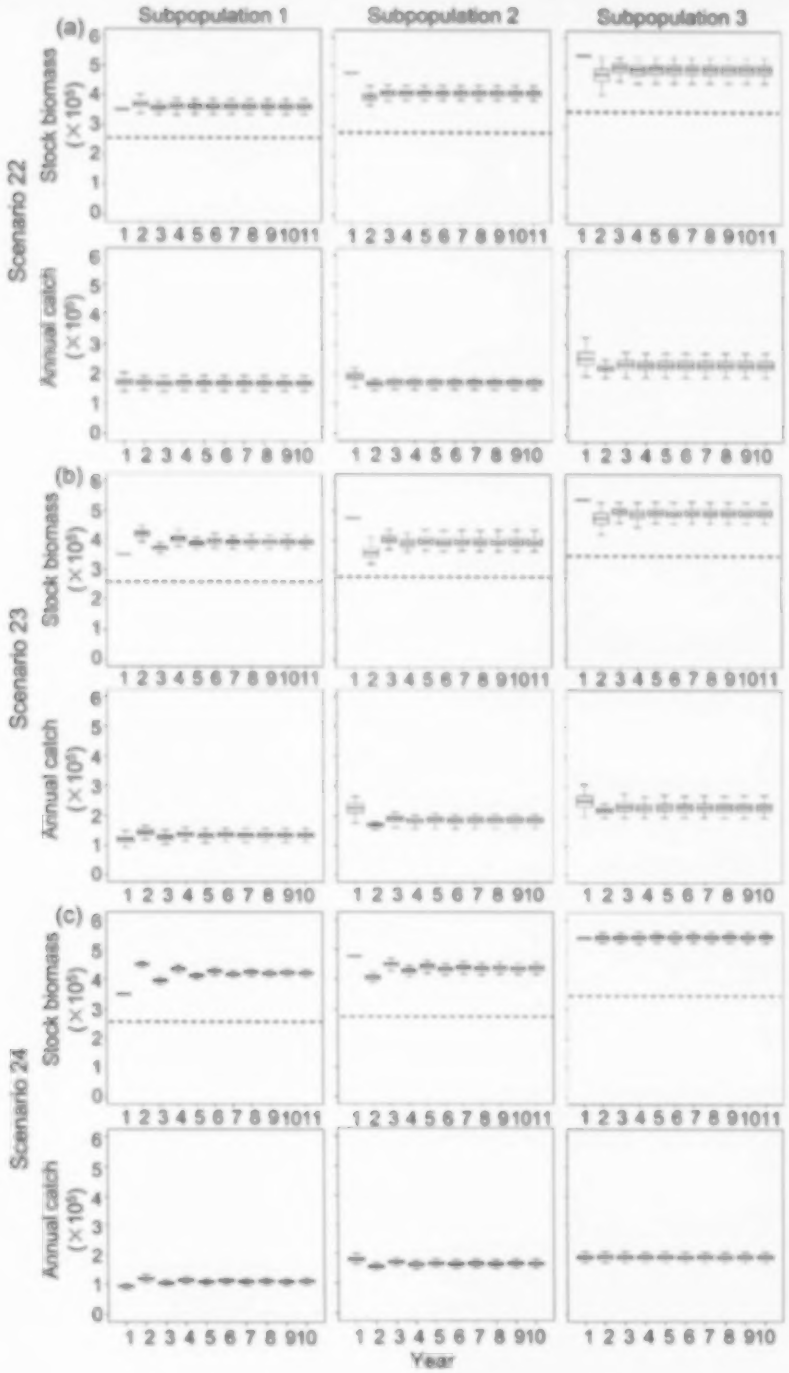
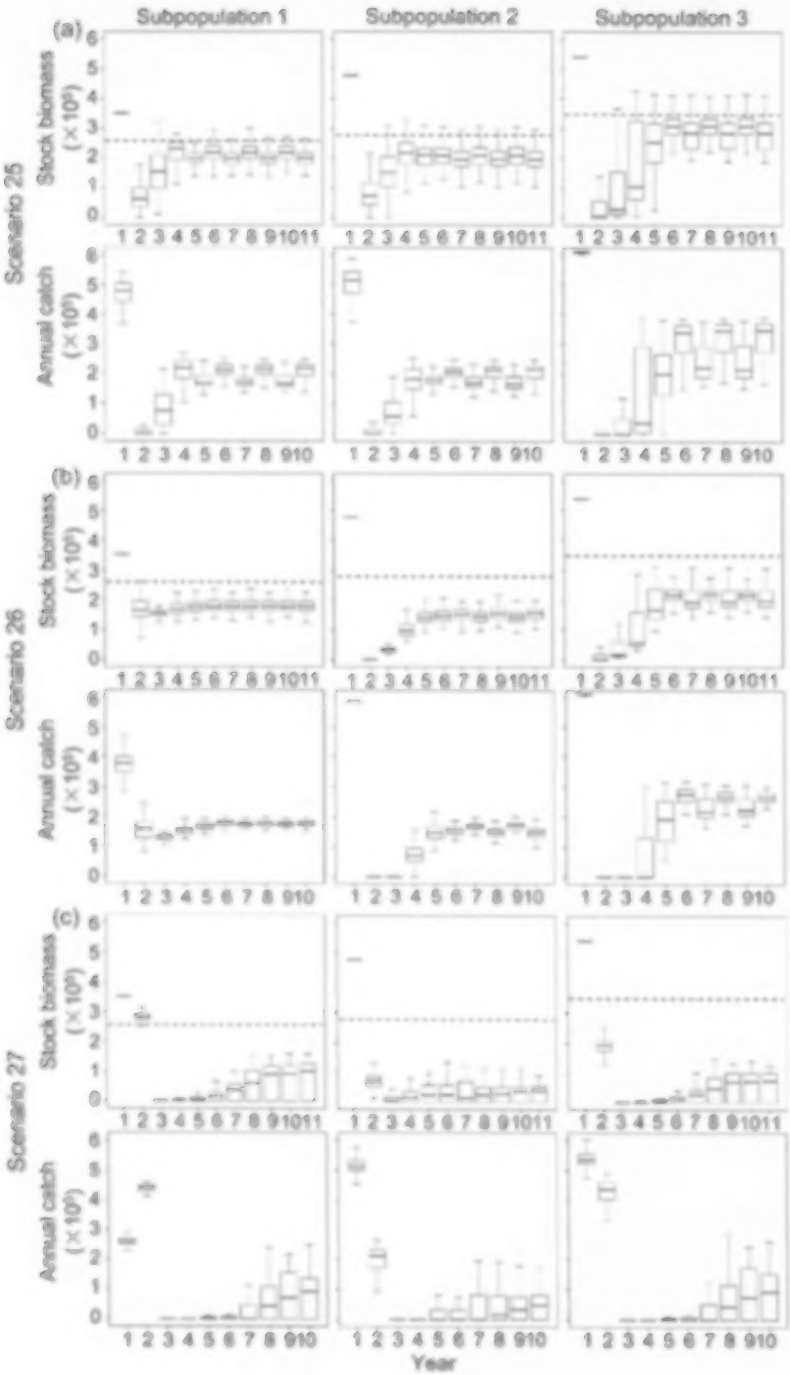


Fig. 13. Boxplots of stock biomass at the beginning of the years and annual catch Scenarios 25 (*a*), 26 (*b*), and 27 (*c*) defined in Table 2 (scenarios with catch level at 1.5MSY for the three spatial options). The biomass management targets of the "true" population are shown by horizontal dotted lines.



a delineation of the population spatial structure and interactions among the subpopulations is critical in developing a sustainable fishery.

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References

Bard, Y. 1974. Nonlinear parameter estimation. Academic Press, New York.

Bell, G. 1990. The ecology and genetics of fitness in *Chlamydomonas*. I. Genotype-by-environment interaction among pure strains. Proc. R. Soc. B Biol. Sci. **240**(1298): 295–321. doi:10.1098/rspb.1990.0039.

Botsford, L.W., Brumbaugh, D.R., Grimes, C., Kellner, J., Largier, J., O'Farrell, M.R., Ralston, S., Soulanille, E., and Wespestad, V. 2009. Connectivity, sustainability, and yield: bridging the gap between conventional fisheries management and marine protected areas. Rev. Fish Biol. Fish. **19**(1): 69–95. doi:10.1007/s11160-008-9092-z.

Chen, Y. 1997. A comparison study of age- and length-structured yield-per-recruit models. Aquat. Living Resour. **10**(5): 271–280. doi:10.1051/alr:1997030.

Chen, Y. 2003. Quality of fisheries data and uncertainty in stock assessment. Sci. Mar. **67**(Suppl. 1): 75–87.

Chen, Y., and Montgomery, S.S. 1999. Modeling the dynamics of eastern rock lobster, *Jasus verreauxi*, stock in New South Wales, Australia. Fish Bull. **97**: 25–38.

Chinese Fishery Yearbook Publishing House. 2000–2009. China Fishery Yearbook (2000–2009). China Agriculture Press, Beijing. [In Chinese.]

Curtis, J.M.R., and Naujokaitis-Lewis, I. 2008. Sensitivity of population viability to spatial and nonspatial parameters using GRIP. Ecol. Appl. **18**(4): 1002–1013. doi:10.1890/07-1306.1. PMID:18536258.

Dichmont, C.M., Deng, A., Punt, A.E., Venables, W., and Haddon, M. 2006. Management strategies for short-lived species: the case of Australia's northern prawn fishery: 1. Accounting for multiple species, spatial structure and implementation uncertainty when evaluating risk. Fish. Res. **82**(1–3): 204–220. doi:10.1016/j.fishres.2006.06.010.

Dieckmann, U., O'Hara, B., and Weisser, W. 1999. The evolutionary ecology of dispersal. Trends Ecol. Evol. **14**(3): 88–90. doi:10.1016/S0169-5347(98)01571-7.

Frank, K.T., and Brickman, D. 2000. Allee effects and compensatory population dynamics within a stock complex. Can. J. Fish. Aquat. Sci. **57**(3): 513–517. doi:10.1139/f00-024.

Han, Z., Lin, L., Shui, B., and Gao, T. 2009. Genetic diversity of small yellow croaker *Larimichthys polyactis* revealed by AFLP markers. Afr. J. Agr. Res. **4**(7): 605–610.

Hanski, I. 1999. Metapopulation ecology. Oxford University Press, Oxford, UK.

Hansson, L. 1991. Dispersal and connectivity in metapopulations. Biol. J. Linn. Soc. **42**(1-2): 89–103. doi:10.1111/j.1095-8312.1991.tb00553.x.

Hart, D.R., and Cadrin, S. 2004. Yellowtail flounder (*Limanda ferruginea*) off the northeastern United States: implications of movement among stocks. *In* Species conservation and management: case studies. *Edited by* H.R. Akçakaya, M. Burgman, O. Kindvall, C.C. Wood, P. Sjögren-Gulve, J.S. Hatfield, and M.A. McCarthy. Oxford University Press, New York. pp. 230–243.

Heath, M.R., Kunzlik, P.A., Gallego, A., Holmes, S.J., and Wright, P.J. 2008. A model of meta-population dynamics for North Sea and West of Scotland cod — the dynamic consequences of natal fidelity. Fish. Res. **93**(1–2): 92–116. doi:10.1016/j.fishres.2008.02.014.

Heifetz, J., and Quinn, T.J., II. 1998. Dynamics of an age by region fish population model. *In* Proceedings of the Symposium on Fishery Stock Assessment Models in the 21st Century, Anchorage, Alaska, 8–11 October 1997. Alaska Sea Grant College Program, Fairbanks, Alaska. pp. 219–244.

Hilborn, R., and Walters, C. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Hutchings, J. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**(5): 943–962. doi:10.1139/f96-097.

Hutchings, J.A. 2000. Collapse and recovery of marine fishes. Nature, **406**(6798): 882–885. doi:10.1038/35022565. PMID:10972288.

Hutchinson, W.F. 2008. The dangers of ignoring stock complexity in fishery management: the case of the North Sea cod. Biol. Lett. **4**(6): 693–695. doi:10.1098/rsbl.2008.0443. PMID:18782730.

Ikeda, I. 1964. Studies on the fisheries biology of the yellow croaker in the East China and the Yellow Seas. Bull. Seikai Reg. Fish. Res. Lab. **31**: 1–81.

Kinlan, B.P., Gaines, S.D., and Lester, S.E. 2005. Propagule dispersal and the scales of marine community process. Divers. Distrib. **11**(2): 139–148. doi:10.1111/j.1366-9516.2005.00158.x.

Kritzer, J.P., and Sale, P.F. 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish Fish. **5**: 131–140.

Li, N., Zhao, S., and Wasiliev, B. 2000. Geology of Marginal Sea in the Northwest Pacific. Heilongjiang Education Press, Harbin, China. [In Chinese.]

Lin, L. 2009. Study on the fishery biology and management strategy of *Larimichthys polyactis* in the southern Yellow Sea and the East China Sea. Ph.D. dissertation, Fisheries College, Ocean University of China, Qingdao, China. [In Chinese with abstract in English.]

Lin, L., Wang, X., and Ma, C. 2006. A preliminary study on total allowable catch (TAC) of *Pseudosciaena polyactis* in the East China Sea Region. Mar. Environ. Sci. **25**: 30–32. [In Chinese with abstract in English.]

Lin, L., Cheng, J., Jiang, Y., Yuan, X., Li, J., and Gao, T. 2008. Spatial distribution and environmental characteristics of the spawning grounds of small yellow croaker in the southern Yellow Sea and the East China Sea. Acta Ecol. Sin. **28**: 3485–3494. [In Chinese with abstract in English.]

Lin, L., Ying, Y., Han, Z., Xiao, Y., and Gao, T. 2009. AFLP analysis on genetic diversity and population structure of small yellow croaker *Larimichthys polyactis*. Afr. J. Biotechnol. **8**: 2700–2706.

Lindenmayer, D.B., Ball, I., Possingham, H.P., McCarthy, M.A., and Pope, M.L. 2001. A landscape-scale test of the predictive ability of a spatially explicit model for population viability analysis. J. Appl. Ecol. **38**(1): 36–48 . doi:10.1046/j.1365-2664.2001.00558.x.

Liu, X. 1962. The research of small yellow croaker (*Larimichthys polyactis*) geographic race and gonad. Science Press, Beijing, China. [In Chinese.]

Mchich, R., Charouki, N., Auger, P., Raissi, N., and Ettahiri, O. 2006. Optimal spatial distribution of the fishing effort in a multi fishing zone model. Ecol. Model. **197**(3–4): 274–280. doi:10.1016/j.ecolmodel.2006.03.026.

Milton, D.A., Rawlinson, N.J.F., and Blaber, S.J.M. 1996. Recruitment

patterns and factors affecting recruitment of five species of short-lived clupeoids in the tropical South Pacific. Fish. Res. **26**(3–4): 239–255. doi:10.1016/0165-7836(95)00434-3.

Orensanz, J.M., Armstrong, J., Armstrong, D., and Hilborn, R. 1998. Crustacean resources are vulnerable to serial depletion — the multifaceted decline of crab and shrimp fisheries in the Greater Gulf of Alaska. Rev. Fish Biol. Fish. **8**(2): 117–176. doi:10.1023/A:1008891412756.

Punt, A.E. 1993. The comparative performance of production-model and ad hoc tuned VPA based feedback-control management procedures for the stock of Cape hake off west coast of South Africa. *In* Risk evaluation and biological reference points for fisheries management. *Edited by* S.J. Smith, J.J. Hunt, and D. Rivard. Can. J. Fish. Aquat. Sci. Spec. Publ. 120. pp. 283–299.

Quinn, T.J., II, and Deriso, R.B. 1999. Quantitative fishery dynamics. Oxford University Press, New York.

Sawchik, J., Dufrene, M., Lebrun, P., Schtickzelle, N., and Baguette, M. 2002. Metapopulation dynamics of the bog fritillary butterfly: modeling the effect of habitat fragmentation. Acta Oecol. **23**(5): 287–296. doi:10.1016/S1146-609X(02)01157-8.

Schtickzelle, N., Choutt, J., Goffart, P., Fichefet, V., and Baguette, M. 2005. Metapopulation dynamics and conservation of the marsh fritillary butterfly: population viability analysis and management options for a critical endangered species in Western Europe. Biol. Conserv. **126**(4): 569–581. doi:10.1016/j.biocon.2005.06.030.

Seikai National Fisheries Research Institute. 2001. Biological and ecological characteristics of valuable fisheries resources from the East China Sea and the Yellow Sea — comparison between the Chinese and Japanese knowledges. Seikai National Fisheries Research Institute, Nagasaki, Japan. [In Japanese.]

Shanks, A.L., Grantham, B.A., and Carr, M.H. 2003. Propagule dispersal distance and the size and spacing of marine reserves. Ecol. Appl. **13**(Suppl. 1): S159–S169. doi:10.1890/1051-0761(2003)013[0159:PDDATS]2.0.CO;2.

Stephenson, R.L. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fish. Res. **43**(1–3): 247–249. doi:10.1016/S0165-7836(99)00076-4.

Trakhtenbrot, A., Nathan, R., Perry, G., and Richardson, D.M. 2005. The importance of long-distance dispersal in biodiversity conservation. Divers. Distrib. **11**(2): 173–181. doi:10.1111/j.1366-9516.2005.00156.x.

Underwood, N. 2007. Variation in and correlation between intrinsic rate of increase and carrying capacity. Am. Nat. **169**(1): 136–141. doi:10.1086/509942. PMID:17206592.

Wilson, J., Ostrom, E., Low, B., and Costanza, R. 1999. Scale misperceptions: and the spatial dynamics of a social–ecological system. Ecol. Econ. **31**(2): 243–257. doi:10.1016/S0921-8009(99)00082-8.

Wooster, W.S. 1992. King crab dethroned. *In* Climate, variability, climate change and fisheries. *Edited by* M.H. Glantz. Cambridge University Press, New York. pp. 14–30.

Influence of environmental stress on age- and size-at-maturity: genetic and plastic responses of coastal marsh fishes to changing salinities

Shannon B. Martin and Paul L. Leberg

Abstract: As sea levels rise, salt water will intrude into freshwater coastal habitats with greater frequency and stress resident organisms inducing physiological trade-offs that influence life history tactics. Western mosquitofish (*Gambusia affinis*) and least killifish (*Heterandria formosa*), collected along a salinity gradient and maintained in a common freshwater environment for two years (≥4 generations), were used to examine historical (genetic effects) and contemporary environmental effects of salinity on age- and size-at-maturity. There was phenotypic plasticity for sexual maturation and genetic variance among source populations for size-at-maturity, but no genetic variance for plasticity itself as indicated by the lack of genetic-by-environment interactions. *Gambusia affinis* males and *H. formosa* females exhibited stress by maturing at smaller sizes and older ages in response to being reared in 0 and 12 ppt, respectively. Our results suggest that habitats in which these fishes are most abundant do not correspond to the salinities at which they can rapidly mature at larger sizes, indicating that other environmental factors are also influential to their distribution and abundance along salinity gradients.

Résumé : À mesure que les niveaux de la mer vont augmenter, l'eau de mer va pénétrer plus fréquemment dans les habitats côtiers d'eau douce, causer des stress aux organismes qui y habitent et entraîner des compromis qui influenceront les tactiques des cycles biologiques. Nous avons utilisé des gambusies (*Gambusia affinis*) et des poissons moustiques (*Heterandria formosa*) récoltés le long d'un gradient de salinité et gardés dans un environnement d'eau douce commun pendant deux ans (≥4 générations) afin d'étudier les effets environnementaux historiques (effets génétiques) et contemporains de la salinité sur l'âge et la taille à la maturité. Il existe une plasticité phénotypique de la maturation sexuelle et une variance génétique entre les populations sources en ce qui concerne la taille à la maturité, mais pas de variance génétique de la plasticité elle-même comme l'indique l'absence d'interactions entre la génétique et l'environnement. Les mâles de *Gambusia affinis* et les femelles d'*H. formosa* montrent des signes de stress en atteignant la maturité à des tailles plus petites et à des âges plus avancés respectivement lorsqu'ils sont élevés à des valeurs de 0 et 12 ppt. Nos résultats laissent croire que les habitats dans lesquels ces poissons sont le plus abondants ne correspondent pas aux salinités dans lesquelles ils peuvent atteindre la maturité à des tailles plus grandes, ce qui indique que d'autres facteurs du milieu influencent aussi leur répartition et leur abondance le long des gradients de salinité.

[Traduit par la Rédaction]

Introduction

Examining patterns of energy allocation along an environmental gradient is crucial for understanding the evolution of life history traits in a changing climate. The energetic costs of dealing with environmental stress can encompass a substantial portion of an organism's energy budget, leaving less energy available for other functions (i.e., growth and reproduction; Sibly and Calow 1989; Wedemeyer et al. 1990). One of the central tenets of life history theory is that organisms can obtain only a finite amount of energy and that the energy is allocated to one function at the expense of another, thus creating trade-offs between competing demands (Stearns 1989, 1992; Roff 1992), following a set of priority rules (Jokela and Mutikainen 1995; Zera and Harshman 2001). Resource demands directly related to survival will have precedence over growth and reproduction (Roff 1983; Jokela and Mutikainen 1995; Glazier 2002). Furthermore, the competing demands for energy remaining after basic maintenance requirements have been met create a trade-off between growth and reproduction (Roff 1983; Calow 1985; Stearns 1992).

Populations can respond to environmental change, such as those caused by climate variability, through several mechanisms: phenotypic plasticity (environmental effects), adaptation (genetic effects), and the interaction between genetic and environmental effects (Stearns 1992; Schlichting and Pigliucci 1998; Pigliucci 2001). Past environmental conditions experienced by local populations will influence phenotypic variation such that phenotypic plasticity is expected to occur when populations have experienced unpredictable environmental conditions, while local adaptation is more likely to occur when populations have experienced relatively stable environmental conditions (Levins 1968; Lytle 2001) and re-

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stricted gene flow over several generations (Sultan and Spencer 2002; Kawecki and Ebert 2004). If genetic variation for plasticity is present, then plasticity, too, is subject to selective pressures of environmental changes.

Life history traits such as age- and size-at-maturity are important determinants of lifetime fitness and have direct consequences for local population dynamics (Cole 1954) in terms of fecundity and reproductive success (Stearns 1992). The changes in selection pressures and trade-offs associated with the developmental transition of maturation make these traits sensitive to environmental changes (Plaistow et al. 2004; Taborsky 2006; Berner and Blanckenhorn 2007) and key life history traits to examine in changing climates (Trippel 1995; Roff 2001; de Roos et al. 2006). Optimally, an organism encountering an environmental stressor that results in slower growth should alter its age- and size-at-maturity along a trajectory or reaction norm that maximizes fitness under the given conditions by changing its life history tactics to mature earlier at a smaller size (Stearns and Koella 1986; Berrigan and Koella 1994). These complex adjustments in growth rates and development time are representative of the physiological trade-off between growth and reproduction (Day and Rowe 2002).

Salinity influences the life histories, population dynamics, and distribution of organisms in coastal ecosystems (Dunson and Travis 1991; Peterson and Meador 1994; Gelwick et al. 2001). With climate change, the increase in the number of extreme disturbance events (e.g., storm surges from tropical storms: Webster et al. 2005), along with the rise in relative sea levels, will allow salt water to intrude into historically freshwater habitats with higher frequency and intensity. Populations of resident marsh fishes that are exposed to these salinity modifications may experience salinity stress, which has deleterious effects on reproductive function and capacity in fishes (Schreck et al. 2001; Wingfield and Sapolsky 2003) by the reallocation of available energy away from reproduction to maintenance for survival. This may not only have a large economic impact because of its effects on coastal fisheries (Bigford 1991), but ecologically it may also affect coastal food webs.

The Louisiana coast is experiencing some of the Earth's highest rates of relative sea level rise (0.9–1.2 cm·year^{-1}; Penland and Ramsey 1990; Gornitz 1995; Donoghue 2011) and salt water intruding into coastal freshwater marshes (Titus and Richman 2001; Nicholls et al. 2007). The western mosquitofish (*Gambusia affinis*) and the least killifish (*Heterandria formosa*) are among the most abundant marsh fishes along the coast of the northern Gulf of Mexico (Hitch et al. 2011). These poeciliids inhabit vegetated margins of fresh to brackish marshes (Meffe and Snelson 1989). Female poeciliids exhibit patterns of indeterminate growth, with the rate of increase slowing exponentially with age after reaching sexual maturity. Conversely, males grow very little after reaching sexual maturity (Hughes 1985; Snelson 1989). Although considered euryhaline (wide salinity tolerance), *G. affinis* and *H. formosa* are most abundant in freshwater habitats (Hitch et al. 2011). However, in a study of natural populations, Martin et al. (2009) found *G. affinis* collected from fresh marsh habitats exhibited indications of environmental stress in the form of lower body condition, reproductive allotment, and fecundity.

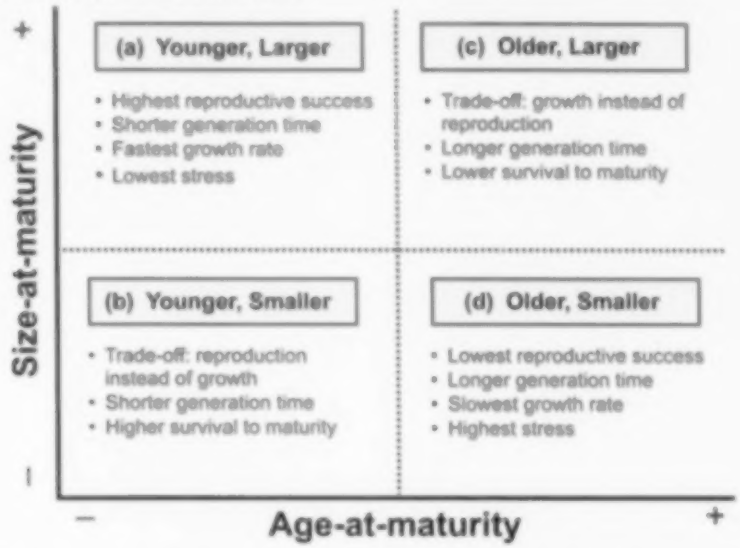
To better understand how coastal marsh fishes might respond to increasing saltwater intrusion from rising sea levels, we assessed the role of salinity in influencing growth and reproduction in *G. affinis* and *H. formosa* by measuring its effect on age- and size-at-maturity. The relative contributions of genetics and the environment to the variation in age- and size-at-maturity and the mechanisms responsible for the trait variation (i.e., adaptation or phenotypic plasticity) were also examined. These species are excellent models to examine these questions because (*i*) they can be collected in high numbers along a salinity gradient, (*ii*) they are small and easily maintained, and (*iii*) they have a short generation time. We hypothesized that energy allocated to growth and reproduction can be reallocated to maintenance in times of environmental stress. The reallocation of resources during development may influence the timing and size at which sexual maturation takes place by influencing the growth rate and (or) developmental timing. Under optimal conditions, we would expect individuals to mature at larger sizes and younger ages to maximize their fitness (Fig. 1). Conversely, in the presence of environmental stress we expect individuals to mature at smaller sizes and older ages, thus impacting their lifetime fitness at the expense of their own current survival (Fig. 1). If historical exposure to a particular salinity level has resulted in localized adaptation, we would expect freshwater fishes to be more susceptible to salinity stress than fish in populations that regularly experience higher salinity levels.

Materials and methods

Sample sites were selected based on plant community structure as described by Visser and colleagues (1998). The different marsh types in this classification system reflect average salinity conditions that occurred in the recent past and are more temporally stable than periodic salinity measurements. This surrogate for environmental salinity was used successfully by Purcell et al. (2008) to investigate local adaptation to salinity in *G. affinis*. In May–September 2004, *G. affinis* and *H. formosa* individuals were collected using a dip net along a salinity gradient from five populations (at least 2 km apart) within each of three marsh types: fresh (0–1 parts per thousand (ppt)), intermediate (1–8 ppt), and brackish (8–15 ppt) in the Terrebonne Basin of southeastern Louisiana (see Martin et al. 2009). Because *H. formosa* rarely occurred in the brackish marsh sites, collections of this species were limited to fresh and intermediate marshes. Fishes collected from the field sites were transported in aerated coolers to an environmentally controlled greenhouse (i.e., 13–35 °C) at the University of Louisiana at Lafayette's Center for Ecology and Environmental Technology (Lafayette, Louuisiana).

The fishes (wild-caught parental generation, P) were separated by species and source population (30 males and 30 females) and placed in large aerated stock tanks containing well water. They were maintained in a "common environment" at a salinity level of 0 ppt for 2 years to remove any potential transgenerational effects (Reznick 1981; Conover and Schultz 1995; Reznick et al. 1996; Weeks and Meffe 1997) from their population's historical salinity exposure in the various source marsh types. Based on an estimate of at least two generations per year (Meffe and Snelson 1989),

Fig. 1. The costs and benefits of the maturation schedule (Stearns 1992, 2000) for a population of organisms divided into four categories based on the intersection of the grand means of age-at-maturity and size-at-maturity. Based on life history theory, predictions can be made on the relative fitness advantages and disadvantages of particular combinations of timing of maturity ((*a*, *b*) younger or (*c*, *d*) older than the mean) and size ((*a*, *c*) larger and (*b*, *d*) smaller than the mean).



this was sufficient time for at least four generations to be reared in common salinity of 0 ppt. Fish were fed daily ad libitum Tetramin flakes and supplemented with *Artemia* nauplii. By maintaining the fish in this controlled environment, we were able to remove potential confounding effects (Sibly 1999) from natural variation in environmental factors such as predation, food level, population density, dissolved oxygen concentrations, pH, water temperature, and salinity.

The maturation experiments examining *H. formosa* were conducted between September and December 2006, and experiments examining *G. affinis* were conducted between April and June 2007. Pregnant females (5 *G. affinis* and 10 *H. formosa* females per source population) were removed from stock populations representing each source marsh type and isolated to give birth. The day the offspring were born, we placed individual offspring in an isolated 2 L container in one of three rearing environments (0, 6, or 12 ppt salinity) using a full cross-factorial design to produce nine treatment combinations of marsh type of the source population and rearing environment (3 × 3) for *G. affinis* and six combinations (2 × 3) for *H. formosa*. This experimental design allowed us to examine the influence of historical (ancestral marsh types of the source populations) and contemporary environmental effects (rearing salinity levels corresponding to the three marsh types) on variation in age and size at sexual maturity of two coastal marsh fish species due solely to environmental salinity levels.

The offspring were raised in isolation to control for social effects on growth and maturity (Hughes 1985; Campton and Gall 1988*b*; Snelson 1989). The assignment of offspring was not random to ensure that siblings were separated equally among the salinity treatments. We fed the offspring *Artemia* nauplii ad libitum daily. After a 2-week period, individuals were assessed daily for maturity. The criteria for sexual maturity of males was the presence of a fully formed gonopodium (Turner 1941), while for females it was the presence of an anal spot (i.e., dark concentration of melanophores appearing above the gonoduct; Farr and Travis 1986; Constantz 1989). When an individual reached maturity, it was euthanized with MS-222 and standard length was measured to the nearest 0.01 mm using digital calipers. Age-at-maturity was calculated as the number of days between birth and sexual maturation.

Statistical analyses

All statistical analyses were conducted using Proc Mixed in SAS Enterprise Guide 4.1 (SAS Institute Inc. 2007), and all higher-order interactions were removed from the multilevel models if they were not significant ($\alpha = 0.05$). Data were separated by species and sex and analyzed using a mixed-model nested analysis of covariance (ANCOVA). Standard length-at-maturity was the dependent variable, source populations were nested within marsh types, family was nested within source population, and the rearing salinity treatments were used as the independent variable, with age-at-maturity as a covariate (Littell et al. 2006). The sample sizes for each treatment combination were unequal owing to unknown offspring sex ratios at birth and some, albeit minimal, mortality. Interactions between age-at-maturity and both source population and rearing environment were assessed to determine if the slope of the relationship between size- and age-at-maturity was the same across treatments.

Within the analysis of variance (ANOVA) and ANCOVA statistical framework, the phenotypic variances of age- and size-at-maturity were partitioned into the main effects of (*i*) the genetic effects due to different source populations and (*ii*) the environmental effects due to the rearing salinity levels, in addition to (*iii*) the interaction of the source marsh

Table 1. Means and standard errors for both age- and size-at-maturity at each level of source marsh type (F, fresh; I, intermediate; B, brackish marshes) and rearing salinity (0, 6, and 12 ppt) and the comparisons of treatments.

	Age-at-maturity (days)		Size-at-maturity (mm)	
Treatment	Male	Female	Male	Female
Heterandria formosa				
	(N = 237)	(N = 339)	(N = 237)	(N = 339)
F	52.18±1.84	40.40±0.94	10.61±0.06	9.65±0.11
I	53.01±2.65	37.55±1.22	10.61±0.06	9.82±0.12
	F ≈ I	**F > I**	**F ≈ I**	**F ≈ I**
0	50.27±2.14	38.03±1.24	10.05±0.12	10.05±0.12
6	54.26±2.52	37.33±1.08	9.75±0.13	9.75±0.13
12	55.61±4.15	43.82±1.71	9.46±0.17	9.46±0.17
	0 ≈ 6 ≈ 12	**0 ≈ 6 < 12**	**0 ≈ 6 ≈ 12**	**0 > 6 > 12**
Gambusia affinis				
	(N = 632)	(N = 578)	(N = 632)	(N = 578)
F	22.42±0.21	31.41±0.27	13.57±0.08	16.18±0.10
I	22.85±0.20	31.40±0.23	13.26±0.07	15.94±0.10
B	22.58±0.19	29.77±0.21	13.30±0.07	15.62±0.08
	F ≈ I ≈ B	**F ≈ I > B**	**F > I ≈ B**	**F > I ≈ B**
0	22.99±0.20	30.96±0.24	13.23±0.07	15.78±0.09
6	22.32±0.21	30.20±0.23	13.53±0.08	16.04±0.09
12	22.51±0.20	31.17±0.24	13.39±0.07	15.80±0.09
	0 > 6 ≈ 12	**0 ≈ 12 > 6**	**0 < 6**	**0 ≈ 12 < 6**

types and experimental rearing salinities (i.e., G × E interaction) (Pigliucci 2001; Conner and Hartl 2004). This division was used to test hypotheses concerning the mechanisms of variation in age- and size-at-maturity in *G. affinis* and *H. formosa*: (1) genetic differences alone, (2) phenotypic plasticity, and (3) genetic variation in plasticity.

Results

There were no significant interactions between the ancestral marsh type of source populations and experimental rearing salinity for any of the life history parameters analyzed for *H. formosa* and *G. affinis*. We did find significant main effects due to the ancestral marsh type of the source populations and experimental rearing salinity for both age- and size-at-maturity for female *H. formosa* and male and female *G. affinis* (Table 1).

Heterandria formosa

We did find that both age- and size-at-maturity were positively correlated in male *H. formosa* (r = 0.495, P < 0.001), age-adjusted size-at-maturity differed between source populations within marsh types ($F_{[8,140]}$ = 2.45, P = 0.017), and size-adjusted age-at-maturity differed between families within source populations ($F_{[74,148]}$ = 1.60, P = 0.008). However, there were no statistically significant differences detected for age-at-maturity and size-at-maturity of male *H. formosa* (N = 237) across source marsh types and rearing environments utilizing ANCOVA. Across all treatments, the grand mean age-at-maturity for *H. formosa* males was 53.8 days (range of 17–90 days), and the grand mean standard length at maturity was 10.6 mm (range of 9.1–12.5 mm).

For *H. formosa* females, there was a significant relationship between age- and size-at-maturity among the rearing environments ($F_{[2,235]}$ = 3.26, P = 0.04). The slope was much greater in the intermediate rearing salinity than in the lower and higher salinities, for which the slopes were slightly positive and negative, respectively (Fig. 2). Size-at-maturity increased with age at 6 ppt, whereas the association between size- and age-at-maturity was less pronounced in the other two rearing salinities. We detected significant interpopulation variation for size-at-maturity ($F_{[8,235]}$ = 3.23, P = 0.002) in *H. formosa* females, but not for age-at-maturity ($F_{[2,327]}$ = 1.69, P = 0.099). However, *H. formosa* females from the ancestral fresh marsh source matured at older ages compared with those from intermediate marshes ($F_{[1,166]}$ = 10.99, P = 0.001; Table 1), while they matured at a similar sizes ($F_{[1,171]}$ = 2.36, P = 0.127; Fig. 3). Females reared at 12 ppt reached maturity at an older age than did those reared at 0 or 6 ppt salinity ($F_{[2,237]}$ = 9.34, P = 0.001; Fig. 3). Size-at-maturity of *H. formosa* females decreased as rearing salinity increased ($F_{[2,237]}$ = 7.47, P = 0.001; Fig. 3).

Gambusia affinis

There were significant effects of the source marsh type on size-at-maturity with age-at-maturity as a covariate in *G. affinis* males ($F_{[2,612]}$ = 7.97, P < 0.001). The relationships between size- and age-at-maturity had significantly different slopes for fish from the three marsh types. Males maturing at young ages did so at similar sizes regardless of source salinity (Fig. 4). As age-at-maturity increased, male fish descendent from source populations collected from fresh marsh environments increasingly matured at larger sizes than males from intermediate or brackish marsh source populations (Fig. 4). In males, both age- and size-at-maturity varied significantly among source populations ($F_{[12,615]}$ = 23.59, P < 0.001; $F_{[12,612]}$ = 5.95, P < 0.001, respectively; Table 1). Male *G. affinis* from the three source marsh types matured

Fig. 2. The relationships between age- and size-at-maturity of *Heterandria formosa* females raised in 0 (open circles with solid line), 6 (grey circles with long dashed line), and 12 (black circles with short dashed line) parts per thousand salinity treatments (N = 135, 126, and 78, respectively).

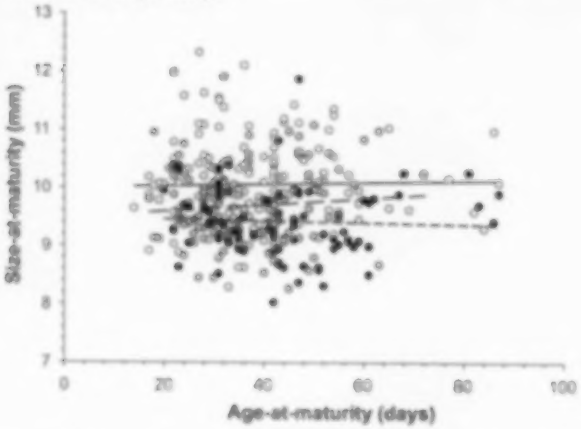
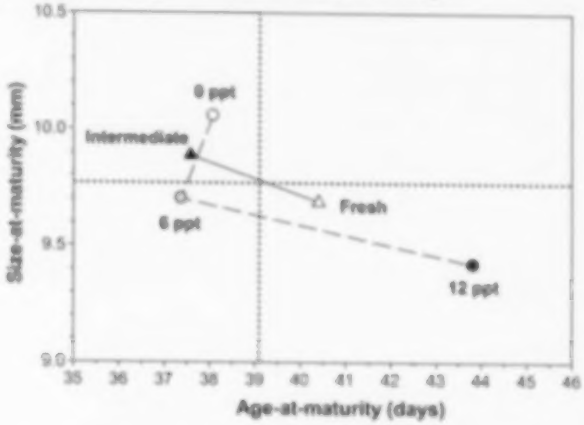


Fig. 3. The bivariate plot of the mean age- and size-at-maturity in *Heterandria formosa* females from ancestral fresh (open triangle) and intermediate sources (black triangle) and reared in the salinity treatments of 0 (open circle), 6 (grey circle), and 12 (black circle) parts per thousand. The two intersecting dotted lines represent the grand means of age-at-maturity and size-at-maturity.



at similar ages ($F_{[2,12]}$ = 0.13, P = 0.881; Fig. 5*a*), but those from fresh marsh source populations matured at larger sizes ($F_{[2,552]}$ = 5.63, P = 0.004; Fig. 5*a*). When raised in fresh water, males matured at older ages ($F_{[2,615]}$ = 4.25, P = 0.015; Fig. 5*a*) and smaller sizes ($F_{[2,612]}$ = 7.80, P = 0.001; Fig. 5*a*) compared with those reared in the higher salinity environments.

For *G. affinis* females, we detected significant effects for both the source marsh type and the salinity treatment for size-at-maturity with age-at-maturity as a covariate. In the ANCOVA, there were no significant interactions between the source marsh type or salinity treatment and age-at-maturity on size-at-maturity ($F_{[2,556]}$ = 1.21, P = 0.298; $F_{[2,556]}$ = 0.74, P = 0.4792, respectively). However, the main effects of source marsh type, salinity treatment, and age-at-maturity on size-at-maturity were statistically significant ($F_{[2,14]}$ = 4.36, P = 0.034; $F_{[2,560]}$ = 5.39, P = 0.005; $F_{[1,560]}$ = 28.09, P < 0.001, respectively; Table 1). We also found significant variation for both age- and size-at-maturity among source populations ($F_{[12,560]}$ = 5.21, P < 0.001; $F_{[12,560]}$ = 2.20, P = 0.011, respectively). Females from the ancestral brackish marsh source populations matured at a younger age compared with those from fresh and intermediate marshes, while the size-at-maturity for females decreased significantly from fresh to intermediate to brackish marshes (Fig. 5*b*). In the 6 ppt rearing salinity treatment, *G. affinis* females matured at a younger age and larger size compared with females exposed to both the 0 and 12 ppt treatments (Fig. 5*b*).

Discussion

Surprisingly, the variation in age- and size-at-maturity in *G. affinis* and *H. formosa* was attributable to both genetic and environmental variation, but there was no interaction between the genetic background of a population and its rearing environment. We were able to detect environmental stress in these common freshwater fishes along a gradient of rearing salinities expressed in the phenotypic plasticity of sexual maturation. Stressed fish slowed growth and delayed sexual development by maturing at older ages and smaller sizes. These results indicate an apparent reallocation of energy to maintenance at the expense of growth and reproduction in response to changes in environmental salinity.

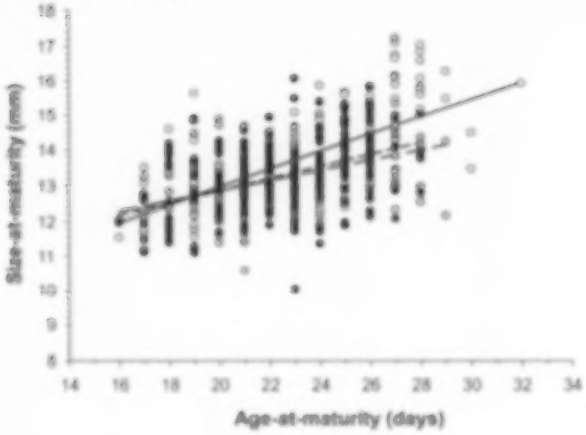
Genetic effects: differences among source populations

Differences in the historical exposure to salinity (ancestral marsh type of source populations) clearly influenced the growth and maturation schedule of *G. affinis* and *H. formosa* during development. Even after 2 years (at least four generations) in a common freshwater environment and the potential for adaptation to the benign laboratory conditions, we were able to detect genetic variation in age- and size-at-maturity based on the differences among source populations within ancestral marsh types (due to past selection under natural conditions). These maturation differences due to source populations must have a genetic component and are not the result of differences in maternal or grandparent environments (Reznick 1981; Reznick et al. 1996; Weeks and Meffe 1997).

Genetic variation in tolerance to environmental stress and its effects on life history traits have been reported in several species of poeciliid fishes. There is genetic variation in thermal stress tolerances within populations of *Gambusia holbrooki* (Meffe et al. 1995) and *H. formosa* (Baer and Travis 2000). It has been previously demonstrated that *H. formosa* has genetic variation in reproductive traits such as offspring size, total number of offspring, and number of broods produced during a female's lifetime (Henrich and Travis 1988).

In our study, differences among the source populations indicated the presence of genetic variation in size-at-maturity for *H. formosa*; however, we did not detect any differences among ancestral source marsh types. We did find that *H. formosa* females from intermediate marshes matured at younger ages and similar sizes compared with those from fresh water. Assuming that favorable environmental conditions result in selection for rapid maturity at larger sizes (Stearns and Koella 1986), for *H. formosa* females such conditions existed

Fig. 4. The relationships between age- and size-at-maturity of *Gambusia affinis* males from fresh (open circles with solid line), intermediate (grey circles with long dashed line), and brackish (black circles with short dashed line) marsh source populations (N = 212, 202, 218, respectively).

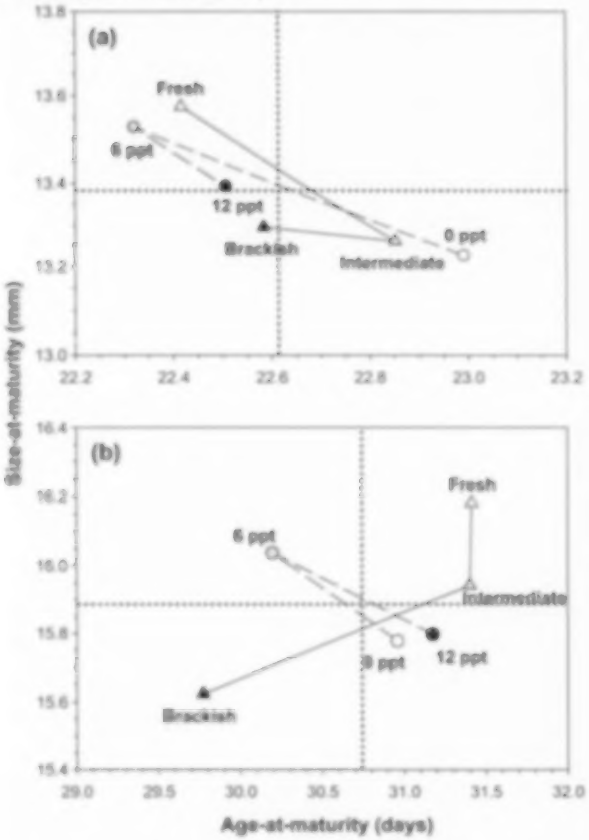


in intermediate marshes rather than in freshwater marshes. We expected fish from source populations collected from freshwater marshes to be adapted for rapid growth and maturation, but that is not what we observed. The lack of any statistical differences detected for the age-at-maturity of *H. formosa* males from fresh and intermediate marshes exposed to the three rearing salinity levels may be due to genetic, physiological, or ecological constraints on growth rates and development time based on their small body size and short life span (Leips et al. 2000).

Age- and size-at-maturity in *G. affinis* have been reported to be highly heritable (Stearns 1983*b*, 1984; Campton and Gall 1988*a*). In our study, *G. affinis* exhibited a significant amount of interpopulation variation in age- and size-at-maturity. We also found significant influences of historical environment of the source populations in *G. affinis* for both age and size-at-maturity. We observed that *G. affinis* males from source populations collected from fresh marshes matured at larger sizes and similar ages compared with males from intermediate and brackish marshes. Unlike males, *G. affinis* females from ancestral fresh marsh source populations matured at older ages and larger sizes.

We detected differences in age- and size-at-maturity based on their source marsh type, but none of those differences were consistent with the prediction of the most optimal phenotype of maturing at younger ages and larger sizes. We focused on salinity as a major contributing factor for our investigation into the variation in age- and size-at-maturity in these fishes. However, the genetic and phenotypic variation in sexual maturation for fishes residing in coastal habitats could be caused by selection pressures from many biotic and abiotic environmental variables influencing coastal marsh communities. Environmental differences in juvenile or adult mortality, resource levels, or density among populations inhabiting the vegetated margins of fresh, intermediate, and brackish marshes as well as other abiotic factors potentially could explain the unexpected results for both *G. affinis* and *H. formosa*.

Fig. 5. The bivariate plot of the mean age- and size-at-maturity in *Gambusia affinis* (*a*) males and (*b*) females from ancestral fresh (open triangles), intermediate (grey triangles), and brackish (black triangles) sources and reared in the salinity treatments of 0 (open circles), 6 (grey circles), and 12 (black circles) parts per thousand. The two intersecting dotted lines represent the grand means of age-at-maturity and size-at-maturity.



In other studies, body size was negatively correlated with population density (Hughes 1985; Snelson 1989; Leips and Travis 1999). Populations of these fishes from higher salinity marshes are much less dense than those from fresh marshes (Hitch et al. 2011). In addition, species composition in submerged aquatic vegetation changes along a salinity gradient in coastal marshes and thus influences habitat complexity, the amount of resources available, and refuge from predators for resident fishes (Rozas and Odum 1988). Both resource availability (Reznick 1990) and predation levels (Reznick et al. 1990; Johnson 2001; Richardson et al. 2006) in source habitats also could be partially responsible for the variation in sexual maturity in *G. affinis* and *H. formosa* descended from populations of fishes collected from different marsh types and account for the differences in population densities along a salinity gradient.

Environmental effects: phenotypic plasticity

Poeciliid fishes are known for their high degree of life history variation and their prevalence of phenotypically plastic

responses in different environments (Trexler 1989) and residing in harsh and capricious habitats (Meffe and Snelson 1989; Nordlie 2006). Phenotypic plasticity is an important mechanism for mitigating the effects of environmental stress. The benefits of phenotypic plasticity are demonstrated in the capacity of an organism to alter its phenotype in changing environments to maximize its fitness in differing conditions (Pigliucci 2001). There are several potential costs to plasticity, such as lower fecundity and the energetic expense of maintaining the sensory and regulatory mechanisms of plasticity (DeWitt et al. 1998). Meffe (1992) reported that mosquitofish can ameliorate the effects of thermal stress by growing slower and maturing at younger ages and smaller body sizes in hot water compared with lower ambient temperatures. In reservoirs in Hawaii, Stearns (1983*a*) found that females matured younger and larger and males matured younger and smaller in *G. affinis* populations inhabiting reservoirs with stable compared with fluctuating water levels. Stearns and Sage (1980) found that *G. affinis* offspring whose parents came from brackish (10 ppt) and freshwater (0 ppt) habitats in Armand Bayou, Texas, performed better when raised in brackish water (10 ppt). They also reported that regardless of their population of origin, *G. affinis* raised in fresh water had lower survival and were smaller at maturity than fish raised in brackish water, demonstrating phenotypic plasticity for several life history traits in environments with different salinity levels (Stearns and Sage 1980). Similarly, *G. affinis* collected from intermediate and brackish marshes (where they were least abundant) exhibited higher body condition and reproductive effort than those mosquitofish from fresh marshes (Martin et al. 2009). It has been demonstrated that *G. affinis* did not vary their marginal metabolic scope when exposed to salinities ranging from 0 to 20 ppt (Akin and Neill 2003). This broad salinity tolerance, coupled with the capacity for phenotypic plasticity in life history traits (such as size- and age-at-maturity), allows this species to occur in capricious and suboptimal habitats.

Although *G. affinis* are considered freshwater fish (Zimmerer 1983), our results suggest that they perform better (i.e., mature younger and larger) in intermediate salinities of 6 ppt. These results are similar to the findings of Stearns and Sage (1980) and Brown-Peterson and Peterson (1990). In addition, Akin and Neill (2003) found that in *G. affinis*, the routine metabolic rate for the species was slightly, but significantly, lower for fish maintained at 10 ppt than for fish acclimated to fresh water. We found that *G. affinis* males reared in the freshwater treatment matured at older ages and smaller sizes, which is a compelling indication of osmotic stress. *Gambusia affinis* females raised in the 6 ppt salinity treatment matured at younger ages and larger sizes than those females in the lowest and highest salinity levels. These results correspond with our findings from the field study in which *G. affinis* exhibited environmental stress in fresh marshes where they were most abundant (Martin et al. 2009). Unlike *G. affinis*, *H. formosa* females displayed the environmental effect predicted for freshwater fishes. *Heterandria formosa* females matured at decreasing sizes when reared in increasing salinity levels. In the highest experimental salinity level of 12 ppt, *H. formosa* females exhibited a strong indicator of salinity stress by maturing at an older age and smaller size and utilizing the life history tactic with the lowest potential fitness.

Lacking G × E interactions

Unexpectedly, we did not detect any significant genetic-by-environment (G × E) interactions (i.e., genetic differences for phenotypic plasticity). The lack of significant interactions between the ancestral marsh type of the source population (used as a proxy for genotypic differences in salinity tolerance) and rearing salinity treatment indicated that while age- and size-at-maturity are both genetically variable and phenotypically plastic in these fishes (as in Stearns and Crandall 1984; Stearns and Koella 1986), there are no distinct differences in plasticity among populations, regardless of their history of past salinity exposure. If populations of these common freshwater fishes were adapted to their source environment, it does not appear to result in their use of the most beneficial life history tactics for sexual maturation in their corresponding rearing environment, such that fishes from habitats with historically higher salinities did not mature at younger ages and larger sizes in more saline-rearing environments.

A genetic basis for population differences in predator-induced phenotypic plasticity in male growth rates and schedule of sexual maturation has been reported in the poeciliid fishes *Poecilia reticulata* (Reznick et al. 1990) and *Brachyrhaphis rhabdophora* (Johnson 2001). Adaptive plasticity has been detected by other investigators as a means of ameliorating the effects of environmental stress through local adaptation in stress tolerance in other organisms (Bernardo 1993, 1994; Johnson 2001; Gomez-Mestre and Tejedo 2003). Although we did not detect any significant G × E interactions (i.e., no differences in plasticity that can be contributed to the ancestral populations exposure to salinity), a major portion of the variation in the traits of age- and size-at-maturity could be explained by the source populations' historical salinity exposure. If these fishes were locally adapted to environmental salinity, then we would expect to find individuals maturing earlier and at larger sizes in a similar salinity from which they were originally collected (which we did not).

Our results suggest that environmental salinity does play a role in the life history variation in *G. affinis* and *H. formosa*. If salinity was the primary driving force for selection on sexual maturation and these fishes were locally adapted to their environmental salinities, we would expect that the plastic response of sexual maturation in rearing environments to match that of the genetic effects from the corresponding source marsh types. Although we found that the plastic responses were different among the rearing salinities, they were consistent across source marsh types (hence no G × E interaction) and did not match with each corresponding source. The lack of G × E interaction and optimal phenotype matching based on our results support the idea that selection pressure from salinity was not the primary factor for the variation in sexual maturation in *G. affinis* and *H. formosa* collected along a salinity gradient of coastal marshes. The differences in the phentotypic patterns between source population and environmental variation denotes a more complex and possibly confounding of biotic environmental variables and community structure (Ghalambor et al. 2007) that may be indirectly related to salinity (Dunson and Travis 1991), such as differen-

ces in resource availability, vegetative cover (Rozas and Odum 1988), predators, and predation pressure between the different marsh types that *G. affinis* and *H. formosa* inhabit (Richardson et al. 2006).

Changing environments

Animals may avoid or minimize the effects of stress by evasion, acclimation, or adaption to their environment. Some coastal populations of freshwater fishes may no longer have the option of evading environmental stress of salt water intruding into freshwater habitats because of the lack of connectivity of their habitat to adjacent, unaffected freshwater systems (not able to migrate from bad habitats (sink) to good habitats (source); sensu Pulliam 1988), leaving the two remaining alternatives (phenotypic plasticity and genetic adaptation) to be used or face local extinction. Purcell and colleagues (2008) reported that *G. affinis* from marsh habitats (same fresh and brackish marsh source populations as this study) with historical exposure to elevated salinity had adapted increased tolerance to lethal levels of salinity stress. Furthermore, the metabolic capacity of adult *G. affinis* varies little over wide range of salinities from 0 to 20 ppt, even though the routine metabolic rate is slightly lower at intermediate salinities (Akin and Neill 2003). Yet, we found that under a narrower range of nonlethal salinities (0–12 ppt), *G. affinis* and *H. formosa* possess significant levels of both genetic and environmental variation in timing and size at sexual maturity with respect to salinity level. Generally, chronic exposure to low salinities has negative impacts on *G. affinis* population size, but some populations inhabiting brackish marsh habitats may develop local adaptations to mitigate the consequences of saltwater intrusion on population demographics (Purcell et al. 2010). An important distinction is that these fishes may have life history traits that have been locally adapted to their source habitats, but not directly to the environmental salinities. Although we detected a significant influence of salinity level on sexual maturation patterns in these fishes, we did not find optimal phenotype matching (local adaptation) between historical salinity levels of source populations and the salinity levels in which these fish were reared with respect to size- and age-at-maturity. While salinity is not likely the sole environmental factor shaping this complex phenotype, it was determined as a source of environmental stress in these coastal marsh fishes.

An important caveat to our findings is that this study, and much of the research on life history responses to salinity in resident marsh fishes, involves small fish with rapid generation times. It is unknown how fish with other life history strategies and salinity tolerances might respond to changes in salinity. It is quite possible that acclimation or movement might become more important in organisms with longer life spans. However, our general observation that knowledge of individual responses to salinity stress will only weakly predict responses to complex natural environments along a salinity gradient is most likely still valid. Additionally, it is worth noting that although such small fishes have a restricted range and represent a portion of life history strategies, they comprise the majority of biomass of the nekton residing in the system (Hitch et al. 2011).

Frequent exposure to disturbances should select for plasticity in organisms, while chronic perturbations or a stable environmental gradient may select for local adaptation (especially when migration is restricted; Sultan and Spencer 2002). The degree of phenotypic plasticity in most life history traits (Caswell 1983; Stearns and Koella 1986; Berrigan and Koella 1994) is expected to increase with greater environmental variation. Having both mechanisms of phenotypic variation could allow populations to deal with environmental fluctuations along a wide range of temporal and spatial scales (Hairston et al. 2005; Gienapp et al. 2008; Visser 2008). The species we studied exhibit both genetic and environmental variation in age- and size-at-maturity because of historical and contemporary exposure to salinity gradients. In addition, individuals within these source populations have the capability to adjust their maturation schedule (phenotypic plasticity) following a similar pattern to maximize their fitness based on short-term, unpredictable environmental fluctuations in salinity during sexual development. The genetic variation in maturation schedule gives coastal fishes the potential to adapt to slow, long-term changes in the environment due to climate change and sea level rise (Gienapp et al. 2008; Purcell et al. 2008, 2010), and the phenotypic plasticity allows them to adjust their life history tactics to deal with short-term stochastic disturbance events (Lytle 2001; Ghalambor et al. 2007), such as saltwater pulses from storm surges of tropical storms. Our results suggest that this scenario is much more complex, in that salinity causes changes in life history traits, but not those expected based on predictions of salinity stress alone. Thus, the consequences of sea level rise on coastal marsh communities may be impossible to predict based solely on the effects of changing salinity because important resident species are responding to a multifaceted environment with changes in both abiotic and biotic factors such as population density, prey, predators, and habitat complexity.

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References

Akin, S., and Neill, W.H. 2003. Routine metabolism of mosquitofish (*Gambusia affinis*) at three different salinities. Tex. J. Sci. **55**(3): 255–262.

Baer, C.F., and Travis, J. 2000. Direct and correlated responses to artificial selection on acute thermal stress tolerance in a livebearing fish. Evolution, **54**(1): 238–244. PMID:10937200.

Bernardo, J. 1993. Determinants of maturation in animals. Trends Ecol. Evol. **8**(5): 166–173. doi:10.1016/0169-5347(93)90142-C. PMID:21236138.

Bernardo, J. 1994. Experimental analysis of allocation in two divergent, natural salamander populations. Am. Nat. **143**(1): 14–38. doi:10.1086/285594.

Berner, D., and Blanckenhorn, W.U. 2007. An ontogenetic perspective on the relationship between age and size at maturity. Funct. Ecol. **21**(3): 505–512. doi:10.1111/j.1365-2435.2007.01253.x.

Berrigan, D., and Koella, J.C. 1994. The evolution of reaction norms: simple models for age and size at maturity. J. Evol. Biol. **7**(5): 549–566. doi:10.1046/j.1420-9101.1994.7050549.x.

Bigford, T.E. 1991. Sea-level rise, nearshore fisheries, and the fishing industry. Coast. Manage. **19**(4): 417–437. doi:10.1080/08920759109362152.

Brown-Peterson, N., and Peterson, M.S. 1990. Comparative life-history of female mosquitofish, *Gambusia affinis*, in tidal freshwater and oligohaline habitats. Environ. Biol. Fishes, **27**(1): 33–41. doi:10.1007/BF00004902.

Calow, P. 1985. Adaptive aspects of energy allocation. *In* Fish energetics: new perspectives. *Edited by* P. Tytler and P. Calow. Johns Hopkins University Press, Baltimore, Md. pp. 13–31.

Campton, D.E., and Gall, G.A.E. 1988*a*. Effect of individual and group rearing on age and size at maturity of male mosquitofish, *Gambusia affinis*. J. Fish Biol. **33**(2): 203–212. doi:10.1111/j.1095-8649.1988.tb05463.x.

Campton, D.E., and Gall, G.A.E. 1988*b*. Responses to selection for body size and age at sexual maturity in the mosquitofish, *Gambusia affinis*. Aquaculture, **68**(3): 221–241. doi:10.1016/0044-8486(88)90355-9.

Caswell, H. 1983. Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences. Am. Zool. **23**(1): 35–46.

Cole, L.C. 1954. The population consequences of life history phenomena. Q. Rev. Biol. **29**(2): 103–137. doi:10.1086/400074. PMID:13177850.

Conner, J.K., and Hartl, D.L. 2004. A primer of ecological genetics. Sinauer Associates, Sunderland, Mass.

Conover, D.O., and Schultz, E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol. Evol. **10**(6): 248–252. doi:10.1016/S0169-5347(00)89081-3. PMID:21237029.

Constantz, G.D. 1989. Reproductive biology of poeciliid fishes. *In* Ecology and evolution of livebearing fishes (Poeciliidae). *Edited by* G. Meffe and F. Snelson. Prentice-Hall, Inc., Englewood Cliffs, N.J. pp. 33–50.

Day, T., and Rowe, L. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. Am. Nat. **159**(4): 338–350. doi:10.1086/338989. PMID:18707419.

de Roos, A.M., Boukal, D.S., and Persson, L. 2006. Evolutionary regime shifts in age and size at maturation of exploited fish stocks. Proc. R. Soc. B Biol. Sci. **273**(1596): 1873–1880. doi:10.1098/rspb.2006.3518. PMID:16822746.

DeWitt, T.J., Sih, A., and Wilson, D.S. 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. **13**(2): 77–81. doi:10.1016/S0169-5347(97)01274-3. PMID:21238209.

Donoghue, J.F. 2011. Sea level history of the northern Gulf of Mexico coast and sea level rise scenarios for the near future. Clim. Change, **107**(1–2): 17–33. doi:10.1007/s10584-011-0077-x.

Dunson, W.A., and Travis, J. 1991. The role of abiotic factors in community organization. Am. Nat. **138**(5): 1067–1091. doi:10.1086/285270.

Farr, J.A., and Travis, J. 1986. Fertility advertisement by female sailfin mollies, *Poecilia latipinna* (Pisces, Poeciliidae). Copeia, **1986**(2): 467–472. doi:10.2307/1445004.

Gelwick, F.P., Akin, S., Arrington, D.A., and Winemiller, K.O. 2001. Fish assemblage structure in relation to environmental variation in a Texas Gulf coastal wetland. Estuaries, **24**(2): 285–296. doi:10.2307/1352952.

Ghalambor, C.K., McKay, J.K., Carroll, S.P., and Reznick, D.N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. **21**(3): 394–407. doi:10.1111/j.1365-2435.2007.01283.x.

Gienapp, P., Teplitsky, C., Alho, J.S., Mills, J.A., and Merilä, J. 2008. Climate change and evolution: disentangling environmental and genetic responses. Mol. Ecol. **17**(1): 167–178. doi:10.1111/j.1365-294X.2007.03413.x. PMID:18173499.

Glazier, D.S. 2002. Resource-allocation rules and the heritability of traits. Evolution, **56**(8): 1696–1700. PMID:12353762.

Gomez-Mestre, I., and Tejedo, M. 2003. Local adaptation of an anuran amphibian to osmotically stressful environments. Evolution, **57**(8): 1889–1899. PMID:14503630.

Gornitz, V. 1995. Sea-level rise: a review of recent past and near-future trends. Earth Surf. Process. Landf. **20**(1): 7–20. doi:10.1002/esp.3290200103.

Hairston, N.G., Jr, Ellner, S.P., Geber, M.A., Yoshida, T., and Fox, J.A. 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecol. Lett. **8**(10): 1114–1127. doi:10.1111/j.1461-0248.2005.00812.x.

Henrich, S., and Travis, J. 1988. Genetic variation in reproductive traits in a population of *Heterandria formosa* (Pisces, Poeciliidae). J. Evol. Biol. **1**(3): 275–280. doi:10.1046/j.1420-9101.1998.1030275.x.

Hitch, A.T., Purcell, K.M., Martin, S.B., Klerks, P.L., and Leberg, P.L. 2011. Interactions of salinity, marsh fragmentation and submerged aquatic vegetation on resident nekton assemblages of coastal marsh ponds. Estuaries Coasts, **34**(3): 653–662. doi:10.1007/s12237-010-9367-1.

Hughes, A.L. 1985. Seasonal trends in body size of adult male mosquitofish, *Gambusia affinis*, with evidence for their social control. Environ. Biol. Fishes, **14**(4): 251–258. doi:10.1007/BF00002628.

Johnson, J.B. 2001. Adaptive life-history evolution in the livebearing fish *Brachyrhaphis rhabdophora*: genetic basis for parallel divergence in age and size at maturity and a test of predator-induced plasticity. Evolution, **55**(7): 1486–1491. PMID:11525470.

Jokela, J., and Mutikainen, P. 1995. Phenotypic plasticity and priority rules for energy allocation in a fresh-water clam: a field experiment. Oecologia, **104**(1): 122–132. doi:10.1007/BF00365570.

Kawecki, T.J., and Ebert, D. 2004. Conceptual issues in local adaptation. Ecol. Lett. **7**(12): 1225–1241. doi:10.1111/j.1461-0248.2004.00684.x.

Leips, J., and Travis, J. 1999. The comparative expression of life-history traits and its relationship to the numerical dynamics of four populations of the least killifish. J. Anim. Ecol. **68**(3): 595–616. doi:10.1046/j.1365-2656.1999.00311.x.

Leips, J., Travis, J., and Rodd, F.H. 2000. Genetic influences on experimental population dynamics of the least killifish. Ecol. Monogr. **70**(2): 289–309. doi:10.1890/0012-9615(2000)070[0289:GIOEPD]2.0.CO;2.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, Princeton, N.J.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., and Schabenberber, O. 2006. SAS system for mixed models. 2nd ed. SAS Institute Inc., Cary, N.C.

Lytle, D.A. 2001. Disturbance regimes and life history evolution. Am. Nat. **157**(5): 525–536. doi:10.1086/319930. PMID:18707260.

Martin, S.B., Hitch, A.T., Purcell, K.M., Klerks, P.L., and Leberg, P.L. 2009. Life history variation along a salinity gradient in coastal marshes. Aquat. Biol. **8**(1): 15–28. doi:10.3354/ab00203.

Meffe, G.K. 1992. Plasticity of life-history characters in eastern mosquitofish (*Gambusia holbrooki*, Poeciliidae) in response to thermal stress. Copeia, **1992**(1): 94–102. doi:10.2307/1446539.

Meffe, G., and Snelson, F. (*Editors*). 1989. An ecological overview of poeciliid fishes. *In* Ecology and Evolution of Livebearing Fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, N.J. pp. 13–31.

Meffe, G.K., Weeks, S.C., Mulvey, M., and Kandl, K.L. 1995. Genetic differences in thermal tolerance of eastern mosquitofish (*Gambusia holbrooki* Poeciliidae) from ambient and thermal ponds. Can. J. Fish. Aquat. Sci. **52**(12): 2704–2711. doi:10.1139/f95-259.

Nicholls, R.J., Wong, P.P., Burkett, V.R., Codignotto, J.O., Hay, J.E., McLean, R.F., Ragoonaden, S., and Woodroffe, C.D. 2007. Coastal systems and low-lying areas. *In* Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited by* M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J.v.d. Linden, and C.E. Hanson. Cambridge University Press, Cambridge, UK. pp. 315–356.

Nordlie, F.G. 2006. Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern North America. Rev. Fish Biol. Fish. **16**(1): 51–106. doi:10.1007/s11160-006-9003-0.

Penland, S., and Ramsey, K.E. 1990. Relative sea-level rise in Louisiana and the Gulf of Mexico: 1908–1988. J. Coast. Res. **6**(2): 323–342.

Peterson, M.S., and Meador, M.R. 1994. Effects of salinity on freshwater fishes in coastal plain drainages in the southeastern U.S. Rev. Fish. Sci. **2**(2): 95–121. doi:10.1080/10641269409388554.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, Md.

Plaistow, S.J., Lapsley, C.T., Beckerman, A.P., and Benton, T.G. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proc. R. Soc. Lond. B Biol. Sci. **271** (1542): 919–924. doi:10.1098/rspb.2004.2682. PMID:15255046.

Pulliam, H.R. 1988. Sources, sinks, and population regulation. Am. Nat. **132**(5): 652–661. doi:10.1086/284880.

Purcell, K.M., Hitch, A.T., Klerks, P.L., and Leberg, P.L. 2008. Adaptation as a potential response to sea-level rise: a genetic basis for salinity tolerance in populations of a coastal marsh fish. Evol. Appl. **1**(1): 155–160. doi:10.1111/j.1752-4571.2007.00001.x.

Purcell, K.M., Klerks, P.L., and Leberg, P.L. 2010. Adaptation to sea level rise: Does local adaptation influence the demography of coastal fish populations? J. Fish Biol. **77**(6): 1209–1218. doi:10.1111/j.1095-8649.2010.02727.x. PMID:21039500.

Reznick, D. 1981. Grandfather effects: the genetics of interpopulation differences in offspring size in the mosquito fish. Evolution, **35**(5): 941–953. doi:10.2307/2407865.

Reznick, D. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. J. Evol. Biol. **3**(3–4): 185–203. doi:10.1046/j.1420-9101.1990.3030185.x.

Reznick, D., Bryga, H., and Endler, J. 1990. Experimentally induced life-history evolution in a natural population. Nature, **346**(6282): 357–359. doi:10.1038/346357a0.

Reznick, D., Callahan, H., and Llauredo, R. 1996. Maternal effects on offspring quality in poeciliid fishes. Am. Zool. **36**(2): 147–156.

Richardson, J., Gunzburger, M., and Travis, J. 2006. Variation in predation pressure as a mechanism underlying differences in numerical abundance between populations of the poeciliid fish *Heterandria formosa*. Oecologia, **147**(4): 596–605. doi:10.1007/s00442-005-0306-y. PMID:16341890.

Roff, D.A. 1983. An allocation model of growth and reproduction in fish. Can. J. Fish. Aquat. Sci. **40**(9): 1395–1404. doi:10.1139/f83-161.

Roff, D.A. 1992. The evolution of life histories. Chapman & Hall, New York.

Roff, D.A. 2001. Age and size at maturity. *In* Evolutionary ecology: concepts and case studies. *Edited by* C.W. Fox, D.A. Roff, and D.J. Fairbairn. Oxford University Press, Oxford, UK. pp. 99–112.

Rozas, L.P., and Odum, W.E. 1988. Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. Oecologia, **77**(1): 101–106. doi:10.1007/BF00380932.

SAS Institute Inc. 2007. SAS eenterprise guide 4.1: SAS OnDemand for academics. SAS Institute Inc., Cary, N.C.

Schlichting, C.D., and Pigliucci, M. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer Associates, Inc., Sunderland, Mass.

Schreck, C.B., Contreras-Sanchez, W., and Fitzpatrick, M.S. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture, **197**(1–4): 3–24. doi:10.1016/S0044-8486(01)00580-4.

Sibly, R.M. 1999. Efficient experimental designs for studying stress and population density in animal populations. Ecol. Appl. **9**(2): 496–503. doi:10.1890/1051-0761(1999)009[0496:EEDFSS]2.0.CO;2.

Sibly, R.M., and Calow, P. 1989. A life-cycle theory of responses to stress. Biol. J. Linn. Soc. **37**(1–2): 101–116. doi:10.1111/j.1095-8312.1989.tb02007.x.

Snelson, F. 1989. Social and environmental control of life history traits. *In* Ecology and evolution of livebearing fishes (Poeciliidae). *Edited by* G. Meffe and F. Snelson. Prentice Hall, Englewood Cliffs, N.J. pp. 149–161.

Stearns, S.C. 1983*a*. The evolution of life-history traits in mosquitofish since their introduction to Hawaii in 1905: rates of evolution, heritabilities, and developmental plasticity. Am. Zool. **23**(1): 65–75.

Stearns, S.C. 1983*b*. The genetic basis of differences in life-history traits among six populations of mosquitofish (*Gambusia affinis*) that shared ancestors in 1905. Evolution, **37**(3): 618–627. doi:10.2307/2408274.

Stearns, S.C. 1984. Heritability estimates for age- and length-at-maturity in two populations of mosquito fish that shared ancestors in 1905. Evolution, **38**(2): 368–375. doi:10.2307/2408495.

Stearns, S.C. 1989. Tradeoffs in life-history evolution. Funct. Ecol. **3**(3): 259–268. doi:10.2307/2389364.

Stearns, S.C. 1992. The evolution of life histories. Oxford University Press, Oxford, UK.

Stearns, S.C. 2000. Life history evolution: successes, limitations, and prospects. Naturwissenschaften, **87**(11): 476–486. doi:10.1007/s001140050763. PMID:11151666.

Stearns, S.C., and Crandall, R.E. 1984. Plasticity for age and size at sexual maturity: a life-history response to unavoidable stress. *In* Fish reproduction: strategies and tactics. *Edited by* G.W. Potts and R.J. Wootton. Academic Press, London, UK. pp. 13–33.

Stearns, S.C., and Koella, J.C. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution, **40**(5): 893–913. doi:10.2307/2408752.

Stearns, S.C., and Sage, R.D. 1980. Maladaptation in a marginal population of the mosquito fish, *Gambusia affinis*. Evolution, **34**(1): 65–75. doi:10.2307/2408315.

Sultan, S.E., and Spencer, H.G. 2002. Metapopulation structure favors plasticity over local adaptation. Am. Nat. **160**(2): 271–283. doi:10.1086/341015. PMID:18707492.

Taborsky, B. 2006. The influence of juvenile and adult environments on life-history trajectories. Proc. R. Soc. Lond. B Biol. Sci. **273**(1587): 741–750. doi:10.1098/rspb.2005.3347. PMID:16608695.

Titus, J.G., and Richman, C. 2001. Maps of lands vulnerable to sea level rise: modeled elevations along the US Atlantic and Gulf coasts. Clim. Res. **18**(3): 205–228. doi:10.3354/cr018205.

Trexler, J.C. 1989. Phenotypic plasticity in poeciliid life histories. *In* Ecology and evolution of livebearing fishes (Poeciliidae). *Edited by* G. Meffe and F. Snelson. Prentice-Hall, Inc., Englewood Cliffs, N.J. pp. 201–214.

Trippel, E.A. 1995. Age at maturity as a stress indicator in fisheries. Bioscience, **45**(11): 759–771. doi:10.2307/1312628.

Turner, C.L. 1941. Morphogenesis of the gonopodium in *Gambusia affinis affinis*. J. Morphol. **69**(1): 161–185. doi:10.1002/jmor.1050690107.

Visser, M.E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. Proc. R. Soc. Lond. B Biol. Sci. **275**(1635): 649–659. doi:10.1098/rspb.2007.0997. PMID: 18211875.

Visser, J., Sasser, C., Chabreck, R., and Linscombe, R. 1998. Marsh vegetation types of the Mississippi River Deltaic Plain. Estuaries, **21**(4B): 818–828. doi:10.2307/1353283.

Webster, P., Holland, G., Curry, J., and Chang, H. 2005. Changes in tropical cyclone number, duration, and intensity in a warming environment. Science, **309**(5742): 1844–1846. doi:10.1126/science.1116448. PMID:16166514.

Wedemeyer, G.A., Barton, B.A., and McLeay, D.J. 1990. Stress and acclimation. *In* Methods for fish biology. *Edited by* C. Schreck and P. Moyle. American Fisheries Society, Bethesda, Md. pp. 451–489.

Weeks, S.C., and Meffe, G.K. 1997. "Grandfather effects" on offspring size in the eastern mosquitofish, *Gambusia holbrooki*. Copeia, **1997**(4): 869–874. doi:10.2307/1447308.

Wingfield, J.C., and Sapolsky, R.M. 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. **15**(8): 711–724. doi:10.1046/j.1365-2826.2003.01033.x. PMID: 12834431.

Zera, A.J., and Harshman, L.G. 2001. The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. **32**(1): 95–126. doi:10.1146/annurev.ecolsys.32.081501.114006.

Zimmerer, E.J. 1983. Effect of salinity on the size-hierarchy effect in *Poecilia latipinna*, *Poecilia reticulata* and *Gambusia affinis*. Copeia, **1983**(1): 243–245. doi:10.2307/1444721.

Response of non-native lake trout (*Salvelinus namaycush*) to 15 years of harvest in Yellowstone Lake, Yellowstone National Park

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Abstract: Introduced lake trout (*Salvelinus namaycush*) threaten to extirpate native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) in the 34 000 ha Yellowstone Lake in Yellowstone National Park, USA. Suppression (and eventual eradication) of the lake trout population is deemed necessary for the conservation of Yellowstone cutthroat trout. A US National Park Service gill-netting program removed nearly 450 000 lake trout from Yellowstone Lake from 1995 through 2009. We examined temporal variation in individual growth, body condition, length and age at maturity, fecundity, mortality, and population models to assess the efficacy of the lake trout suppression program. Population metrics did not indicate overharvest despite more than a decade of fish removal. The current rate of population growth is positive; however, it is lower than it would be in the absence of lake trout suppression. Fishing effort needs to increase above observed levels to reduce population growth rate below replacement. Additionally, high sensitivity of population growth rate to reproductive vital rates indicates that increasing fishing mortality for sexually mature lake trout may increase the effectiveness of suppression. Lake trout suppression in Yellowstone Lake illustrates the complexities of trying to remove an apex predator to restore a relatively large remote lentic ecosystem with a simple fish assemblage.

Résumé : Les touladis (*Salvelinus namaycush*) introduits dans le lac Yellowstone de 34 000 ha dans le parc national de Yellowstone, É.-U., menacent d'en extirper la truite fardée de Yellowstone (*Oncorhynchus clarkii bouvieri*) indigène. On croit que la réduction (et éventuellement l'éradication) de la population de touladis est nécessaire à la conservation des truites fardées de Yellowstone. Un programme de pêche au filet maillant mené par le service des parcs nationaux des É.-U. a retiré presque 450 000 touladis du lac Yellowstone de 1995 jusqu'à la fin de 2009. Nous examinons la variation temporelle de la croissance individuelle, de la condition corporelle, de la longueur et de l'âge à la maturité, de la fécondité, de la mortalité et des modèles démographiques afin d'évaluer l'efficacité du programme d'élimination des touladis. Les métriques de la population n'indiquent pas de récolte excessive malgré le retrait de poissons pendant plus d'une décennie. Le taux actuel de croissance de la population est positif; cependant, il est plus bas qu'il ne le serait en absence du programme d'élimination des touladis. L'effort de pêche devra être accru au-delà des niveaux observés afin de réduire le taux de croissance de la population sous le seuil de remplacement. De plus, la forte sensibilité du taux de croissance de la population aux taux vitaux reproductifs indique que l'augmentation de la mortalité due à la pêche chez les touladis à maturité sexuelle pourrait améliorer l'efficacité de l'élimination. L'élimination des touladis dans le lac de Yellowstone illustre les difficultés reliées aux efforts pour retirer un prédateur de sommet de réseau trophique afin de restaurer un écosystème lénitique relativement grand avec un peuplement simple de poissons.

[Traduit par la Rédaction]

Introduction

Non-native species have long been recognized as a driver of the global decline in biodiversity (Vitousek et al. 1997; Simberloff 2001) and are often ranked second to habitat loss and degradation as the most prevalent threat to freshwater biodiversity (Miller et al. 1989; Cambray 2003; Dextrase and Mandrak 2006). Non-native fishes have been implicated in the decline of many native freshwater fish species worldwide (Holčík 1991; Ogutu-Ohwayo and Hecky 1991; Jelks et al. 2008). Eradication projects have been implemented for populations of non-native fishes; however, peer-reviewed articles assessing the efficacy of eradication projects are sparse, and most of these projects have occurred on small spatial scales such as high mountain streams, alpine lakes, and small impoundments (Thompson and Rahel 1996; Knapp and Mat-

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thews 1998; Britton and Brazier 2006). Information on suppression or eradication of populations of non-native fishes in larger water bodies is becoming increasingly important. For example, the invasion of several carp species (*Hypophthalmichthys* and *Cyprinus* spp.) throughout large river systems in North America and Australia may shift the management focus for these species from containment to removal (Kolar and Lodge 2002; Koehn 2004; DeGrandchamp et al. 2008).

The objective of many fish removal projects is to reverse native fish population declines caused by non-native predators (Mueller 2004; Louette and Declerck 2006; Martinez et al. 2009). The lake trout (*Salvelinus namaycush*) is an apex predator native to northern North America that has been introduced to 15 countries around the world and extensively within the United States (Crossman 1995). Lake trout have negatively affected native fish species in ecosystems where they have been successfully introduced (Crossman 1995), including large lakes and reservoirs in eight western US states (Martinez et al. 2009), where their presence has led to declines in native salmonid populations (Fredenberg 2002; Vander Zanden et al. 2003; Koel et al. 2005). Introductions of lake trout have also altered trophic dynamics in lakes and surrounding terrestrial ecosystems (Vander Zanden et al. 2003; Tronstad et al. 2010; Ellis et al. 2011).

Lake trout were discovered in Yellowstone Lake, Yellowstone National Park, in 1994 (Kaeding et al. 1995) after being illegally introduced in the mid- to late 1980s (Munro et al. 2005). Yellowstone Lake is the largest lake above 2000 m elevation in North America and contains the largest population of nonhybridized Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) in the world (Gresswell and Varley 1988). The Yellowstone cutthroat trout is considered a "sensitive species" by the US Forest Service and Bureau of Land Management and an imperiled species by state agencies in Idaho, Montana, Nevada, Utah, and Wyoming (Gresswell 2009). Yellowstone Lake represents nearly 80% of the remaining lacustrine habitat for Yellowstone cutthroat trout (Gresswell et al. 1994), which is a keystone species in the Greater Yellowstone Ecosystem. Spawning migrations of Yellowstone cutthroat trout have been documented in 68 of Yellowstone Lake's 124 tributaries (Gresswell et al. 1994), representing a substantial source of nutrients for the terrestrial ecosystem (Crait and Ben-David 2006). Thirteen mammal species and 15 bird species have been documented feeding on Yellowstone cutthroat trout (Schullery and Varley 1995).

The level of predation exerted by lake trout is expected to cause the Yellowstone cutthroat trout population to decrease by as much as 60% within 100 years (Stapp and Hayward 2002; Ruzycki et al. 2003). Relative abundance of Yellowstone cutthroat trout in netting assessments declined 11% per year after the discovery of lake trout (Koel et al. 2005). A lake trout removal program was initiated in 1995 with the purpose of reducing lake trout abundance and decreasing predation on Yellowstone cutthroat trout (McIntyre 1995). Lake trout suppression has been implemented or considered for the purpose of conserving native salmonids in several lakes in the Intermountain West of the USA (Martinez et al. 2009). As the longest ongoing lake trout removal project in the Intermountain West, the suppression program on Yellowstone Lake provides a unique case study to evaluate the effectiveness of removing a non-native predator from a large lake.

Lake trout have been exploited throughout their native range where the collapse of several lake trout fisheries indicates that overexploitation is possible (Martin and Olver 1980). The vulnerability of lake trout populations to overharvest is attributed to the species' life-history strategy, which is characterized by relatively slow growth and late age at maturity (Healey 1978*a*). However, lake trout population metrics are strongly influenced by lake size, lake productivity, and diet (Trippel 1993; Shuter et al. 1998; Pazzia et al. 2002).

Exploitation can cause changes in population metrics and an eventual decline in abundance. A high level of harvest causes mortality rates to increase, with compensatory responses becoming evident in population metrics if a reduction in abundance reduces the effects of density dependence (Rose et al. 2001). Individual growth rates typically increase in exploited lake trout populations (Healey 1978*a*; Ferreri and Taylor 1996; Sitar and He 2006). Similarly, lake trout body condition increased in populations where exploitation caused a decline in density (Martin and Olver 1980; McDonald and Hershey 1989). Lake trout have also exhibited reductions in age at first maturity and increases in fecundity when harvested (Healey 1978*a*, 1978*b*; Ferreri and Taylor 1996). Although compensatory responses indicate a decrease in abundance, they can increase the resilience of populations to overharvest (Rose et al. 2001). For example, reduced age at maturity and elevated fecundity can increase the reproductive output of an exploited population (Healey 1978*b*; Trippel 1995).

Suppression was implemented by the National Park Service (NPS) in rapid response to the detection of lake trout (McIntyre 1995), with the goal of removing as many lake trout as possible given limited resources. Abundance was not estimated when suppression was initiated; therefore, harvest rates cannot be used to evaluate the effectiveness of the suppression program. We used available data from 1996 through 2009 to assess whether the suppression program has been successful at overharvesting the lake trout population (i.e., recruitment overfishing). We hypothesized that population metrics such as individual growth, body condition, fecundity, length at maturity, age at maturity, and mortality could be used to determine the efficacy of the suppression program given previous research on exploited lake trout populations (see paragraph above). We then used an age-structured matrix demographic model to simulate several scenarios of fishing mortality and the subsequent effects on population growth. These results provide guidance for the NPS and other natural resource agencies with regard to establishing harvest benchmarks to increase suppression program efficacy and highlight the need for baseline data when initiating non-native species removal.

Materials and methods

Study area

Yellowstone Lake is at an elevation of 2357 m, has a surface area of 34 020 ha, 239 km of shoreline, a mean depth of 48.5 m (Kaplinski 1991), and a maximum depth of 133 m (Morgan et al. 2003). The lake is typically ice-covered from mid-December until late May or early June. Thermal stratification occurs through July and early August, with summer surface water temperature reaching 17 °C and a thermocline

at about 15 m (Koel et al. 2007). Hydrothermal activity occurs throughout the lake, but is concentrated in the West Thumb Basin (Kaplinski 1991) where hydrothermal vents create warmer than lake-average water temperatures.

The lake is categorized as oligo-mesotrophic (Theriot et al. 1997), and the fish assemblage consists of two native species, Yellowstone cutthroat trout and longnose dace (*Rhinichthys cataractae*). Three fish species have been introduced in addition to lake trout: longnose sucker (*Catostomus catostomus*), redside shiner (*Richardsonius balteatus*), and lake chub (*Couesius plumbeus*; Gresswell and Varley 1988).

Data collection

The removal of lake trout began when they were first caught in gill nets in 1994 (Kaeding et al. 1995). Removal intensified as gill nets were used to determine the distribution and population characteristics of lake trout from 1995 through 1998 (Ruzycki 2004). Gill nets were first set for the purpose of maximizing lake trout catch in 1998 and were used primarily for this purpose through 2009. Netting methods (i.e., mesh sizes and locations fished) were most similar from 2001 through 2008. Three different netting strategies were used to remove lake trout from Yellowstone Lake: control netting, spawner netting, and distribution netting (see Ruzycki (2004) for a detailed description of netting methods). Control netting targeted juvenile lake trout (250 to 400 mm total length) and constituted the majority of effort exerted in the suppression program since 1998. Control nets were sinking gill nets with bar-mesh sizes of 25, 32, or 38 mm. Annual effort (1 unit = 100 m of net set for 1 night) in control netting increased from 1145 to 16 725 units from 1998 through 2009, peaking at 28 500 units in 2007. Control nets were fished from ice-off in late May or early June through October.

Spawner netting targeted adult lake trout (>400 mm total length) at known spawning or staging locations. Sinking gill nets had bar-mesh sizes of 38, 44, 51, 57, 64, 70, or 76 mm. Effort increased from 142 to 2600 units from 1996 to 2009. Spawner nets were fished from late August through October and were lifted every 1–3 days to minimize gear saturation and bycatch of Yellowstone cutthroat trout. Night electrofishing was conducted from 2004 to 2007 at shallow spawning areas (primarily in the West Thumb Basin) during peak spawning activity. The amount of annual electrofishing effort varied from 8.67 to 26.25 h total electrofishing time (occurring over 5–14 nights per year) and accounted for 5%–20% of all spawning fish removed (i.e., gill-netting and electrofishing pooled).

Distribution netting consisted of lake-wide sampling of multiple depth strata to collect data on lake trout population demographics and spatial distribution. Distribution netting typically occurred at 16 fixed sites throughout the lake. Each site consisted of two depth strata: a shallow stratum (15–30 m) and a deep stratum (30–50 m). A small-mesh (consisting of 19, 25, 32, 38, 44, and 51 mm bar mesh) and a large-mesh (consisting of 57, 64, 70, 76, and 89 mm bar mesh) experimental gill net were combined to form a gang. A gang was set overnight at each depth stratum by site.

Age, growth, and body condition

Sagittal otoliths were used to estimate lake trout ages through the suppression program (Ruzycki et al. 2003; Syslo 2010). Regressions of annual mean length at age values through time were used to assess changes in individual growth rate because of highly variable sample sizes among years. Mean length at age was estimated for lake trout caught on or later than 1 August to avoid possible biases caused by seasonal growth. Mean length at age was not estimated for years where age classes had fewer than five observations to avoid possible influences of outliers. Regressions of mean length at age through time were estimated for age classes with at least five annual mean length values. The von Bertalanffy (VBF) equation was used to describe growth in length and provided parameters to estimate natural mortality (see below). The NLS procedure in R 2.9.2 was used to estimate values of model parameters using the following equation:

$$(1) \qquad L_t = L_\infty\{1 - \exp[-K(t - t_0)]\}$$

where L_t is length at time t, L_∞ is theoretical maximum length of lake trout in the population, K is the growth coefficient, t is age, and t_0 is age when length theoretically equals 0 mm (Isely and Grabowski 2007).

Body condition was compared between 1998 and 2007 because these years had adequate mass data and represent conditions before and after 10 years of removal. Analysis of covariance (ANCOVA) was used to compare mass–length regression models from 1998 and 2007. Gonadal development over the course of the year can influence mass measurements, especially for female fish (Pope and Kruse 2007). Therefore, the ANCOVA model for mass as a function of total length included categorical predictors for year (1998 or 2007), whether fish were mature (>401 mm) or immature (<400 mm), and sex. Mass and length observations were $\log_e$-transformed prior to model estimation for linearity.

Fecundity and maturity

Fecundity was estimated volumetrically in 1998 (Ruzycki et al. 2003) and gravimetrically in 2006 and 2007 (Syslo 2010). Mass–fecundity relationships were compared by year with ANCOVA to determine whether fecundity changed over the duration of the suppression program.

Maturity was estimated for fish caught in distribution netting to avoid biases caused by netting methods that target immature or spawning mature lake trout. Annual probabilities of 50% maturity at length were estimated by sex using logistic regression for binary response data. Logistic regression models included total length and year as continuous variables. Annual values of length at maturity were converted to ages using year-specific VBF growth models.

Mortality and harvest

Recruitment likely increased through time given the relatively recent date of lake trout introduction. Therefore, mortality was estimated for lake trout caught in control and spawner netting using a statistical catch at age analysis (SCAA). The SCAA model was estimated using catch at age observations for age 2–17 lake trout and gill-netting effort from combined control and spawner netting from 2001 through 2008. Methods followed Haddon (2001) except age-specific fishery selectivity was estimated using a double logistic function (Linton et al. 2007). Natural mortality was es-

timated as an instantaneous rate (M) with the formula provided by Pauly (1980):

$$(2)\quad \log(M) = -0.0066 - 0.279 \cdot \log_{10}(L_\infty) + 0.6543 \cdot \log_{10}(K) + 0.4634 \cdot \log_{10}(T)$$

where M is the instantaneous rate of natural mortality, L_∞ and K are coefficients estimated from the VBF growth equation, and T is mean annual environmental water temperature. Mean annual environmental water temperature was 5.1 °C for Yellowstone Lake (Ruzycki et al. 2003).

Estimates of lake trout mortality were obtained using catch curve regressions for lake trout caught in distribution netting in addition to the SCAA for catch data. Although increasing recruitment may bias catch curve estimates of mortality, the distribution netting data were considered most representative of the population, and general agreement between separate data sets would strengthen conclusions. Length structure of fish caught in gill nets may not represent the length structure of the population because gill nets are selective for certain sizes of fish; therefore, length–frequency data were standardized to account for unequal selectivity and effort among mesh sizes (Syslo 2010). Standardized annual length frequencies were converted to age frequencies using year-specific age–length keys (Isely and Grabowski 2007). Age frequencies were computed for years without age data using the age–length key for the nearest year. Total instantaneous mortality rate (Z) was estimated as the slope of catch curve regressions of the natural logarithm of catch at age on age (Miranda and Bettoli 2007). Total instantaneous mortality (Z) was evaluated through time with an ANCOVA model that included year as a continuous variable. Instantaneous mortality rates were converted to total annual mortality (A) and exploitation (μ; Miranda and Bettoli 2007). Exploitation represents the proportion of the population removed over an interval; therefore, by dividing the annual harvest by μ we recreated estimates of annual lake trout abundances. An extremely small lower 95% confidence interval (CI) for μ resulted from a large standard error estimated for Z in 1998 and created an unrealistic upper 95% CI for reconstructed abundance in 1998. Therefore, the CI for μ used to back-calculate abundance in 1998 was estimated using the standard error for Z from the 1997 catch curve regression. Annual abundances after harvest were computed by subtracting the harvest from the back-calculated abundance, and a model was fit to the abundances for an estimate of population growth rate (λ).

Age-structured population model

Model structure

A female-based Leslie matrix was used to model the lake trout population (Caswell 2001). Age-specific reproductive rates were estimated using the most recent estimates of demographic parameters (Table 1). Although the oldest lake trout observed in Yellowstone Lake was 17 years, maximum age estimates in native populations vary from 20 to 62 years (Martin and Olver 1980). Therefore, the matrix model included age classes 0 to 30 because length at age reached an asymptote at age 30. An age 30+ stage was included so that the life span was not limited to 30 years. We assumed that females produced offspring at age 5 (the minimum observed age of sexual maturity) and also assumed females spawned every year. The matrix model included a prebreeding census, which requires the fertility term to include survival of age-0 fish (S_0) to be counted as age-1 fish the following year (Caswell 2001). Fertility was multiplied by 0.5 to account for half of the offspring being female.

Model parameterization

Length at age was used to predict age-specific values of fecundity (Fec_i) and probability of maturity (P_i). Length at age for females was determined using the VBF growth model for females (where $t_0 = -0.16$, $K = 0.18$, and $L_\infty = 781$). The mean and standard deviation for Fec_i and P_i given length at age i were determined from models of length–fecundity ($\log(\mathrm{Fec}_i) = -15.08 + 3.626 \times \log(\mathrm{length}_i)$) and length–maturity:

$$(3)\quad P_i = \frac{\exp(-21.0 + 0.04 \cdot \mathrm{length}_i)}{1 + \exp(-21.0 + 0.04 \cdot \mathrm{length}_i)}$$

Natural mortality was estimated as an instantaneous rate (M) using VBF parameters for females and converted to annual survival rate for ages 2–30+ in the matrix model with the following equation: $S_i = \exp(-M)$ (Miranda and Bettoli 2007). Natural survival rates have not been estimated for age-0 and age-1 lake trout in Yellowstone Lake; therefore, we used estimates from the native range of the species. Annual natural survival rate was assumed to be 0.0043 for age-0 lake trout (Shuter et al. 1998) and 0.449 for age-1 lake trout (Sitar et al. 1999).

Age-specific instantaneous fishing (F) mortality was modeled as the product of fully selected fishing mortality and age-specific selectivity (Haddon 2001). Instantaneous fishing and natural (M) mortality rates were converted to conditional interval fishing (m) and natural (n) mortality rates with the following equations: $m = 1 - \exp(-F)$ and $n = 1 - \exp(-M)$ (Miranda and Bettoli 2007). Estimates of age-specific survival (S_i) that included both natural and fishing mortality were computed as the complement of total conditional interval mortality ($A = m + n - mn$; Miranda and Bettoli 2007).

The matrix model was not sensitive to starting population size because it did not include density dependence. However, short-term results were sensitive to the starting age frequency. We considered the age frequency from distribution netting to be the most representative of the population in any given year. The starting population size was the SCAA abundance estimate for 2008 distributed among age classes using the age frequency for distribution netting in 2009. The abundance of age-1 lake trout was extrapolated from an exponential decay function fit to the age frequency for ages 2–17. The observed sex ratio was 1:1. Therefore, the abundance estimate was multiplied by 0.5, resulting in 231 481 female lake trout ages 1–17.

Model simulations

Fully selected fishing mortality (F) varied from 0 to 1.5 in increments of 0.1. Each level of F was maintained for 5, 10, and 20 years. Each management scenario was simulated 1000 times using R 2.9.2 (R Development Core Team 2009) to incorporate variation in selectivity and vital rate estimates. The first step of each simulation run was to create a Leslie projection matrix replicate. Vital rates for the matrix were estimated from predicted values of age-specific survival, fecundity, and

Table 1. Mean and standard deviation (in parentheses) of age-specific vital rates used in population simulations for lake trout in Yellowstone Lake, Yellowstone National Park.

Age	S_i in absence of fishing mortality	Sel_i	Total length (mm)	Fec_i	P_i
0	0.0043[a]	0	—	0	0
1	0.449[b]	0	145	0	0
2	0.898	1.000 (0.000)	249	0	0
3	0.898	0.995 (0.016)	335	0	0
4	0.898	0.990 (0.034)	407	0	0
5	0.898	0.984 (0.051)	468	822 (289)	0.097 (0.058)
6	0.898	0.977 (0.068)	519	1373 (221)	0.391 (0.107)
7	0.898	0.969 (0.084)	561	2009 (176)	0.732 (0.116)
8	0.898	0.960 (0.010)	597	2660 (152)	0.901 (0.075)
9	0.898	0.950 (0.114)	627	3309 (148)	0.961 (0.040)
10	0.898	0.940 (0.130)	652	3927 (155)	0.983 (0.022)
11	0.898	0.927 (0.143)	673	4499 (168)	0.991 (0.013)
12	0.898	0.914 (0.157)	690	5020 (182)	0.995 (0.008)
13	0.898	0.900 (0.171)	705	5467 (196)	0.997 (0.005)
14	0.898	0.884 (0.185)	717	5882 (208)	0.998 (0.004)
15	0.898	0.868 (0.200)	728	6223 (218)	0.998 (0.003)
16	0.898	0.851 (0.212)	736	6512 (228)	0.999 (0.002)
17	0.898	0.832 (0.224)	743	6778 (236)	0.999 (0.002)
18	0.898	0.832 (0.224)	749	6982 (242)	0.999 (0.002)
19	0.898	0.832 (0.224)	755	7156 (249)	0.999 (0.002)
20	0.898	0.832 (0.224)	759	7332 (253)	0.999 (0.002)
21	0.898	0.832 (0.224)	762	7440 (257)	0.999 (0.002)
22	0.898	0.832 (0.224)	795	7548 (259)	0.999 (0.002)
23	0.898	0.832 (0.224)	768	7621 (262)	0.999 (0.002)
24	0.898	0.832 (0.224)	770	7694 (265)	0.999 (0.002)
25	0.898	0.832 (0.224)	772	7769 (266)	0.999 (0.002)
26	0.898	0.832 (0.224)	773	7806 (269)	0.999 (0.002)
27	0.898	0.832 (0.224)	774	7881 (270)	0.999 (0.002)
28	0.898	0.832 (0.224)	775	7919 (272	0.999 (0.002)
29	0.898	0.832 (0.224)	776	7956 (272)	0.999 (0.002)
30	0.898	0.832 (0.224)	777	7956 (272)	0.999 (0.002)

Note: Age-specific vital rates are survival (S_i), relative selectivity (Sel_i), fecundity (Fec_i), and probability of maturity (P_i).

[a]Vital rate estimate from Shuter et al. (1998), with standard deviation (SD) equal to 15% of the mean.

[b]Vital rate estimate from Sitar et al. (1999), with SD equal to 15% of the mean.

probabilities of maturity and associated standard deviations (Table 1). The popbio package (Stubben and Milligan 2007) was used to generate vital rate values for each matrix replicate. Vital rate values were generated from beta and stretch beta distributions, which have central tendencies and tails that are restricted to logical parameter values instead of approaching infinity (Morris and Doak 2002). Age-specific rates of survival and probabilities of maturity were generated from beta distributions, which had tails restricted between 0 and 1. Age-specific selectivity and fecundity values were log-normally distributed and generated from stretched beta distributions, which had tails restricted to specified minimum and maximum values (Morris and Doak 2002). Each matrix replicate ($\mathbf{A}_t$) was iterated with the population vector ($\boldsymbol{n}_t$) for 5, 10, or 20 years. For each simulation, λ at each time step (λ_t) was computed from the abundance (N_t): $\lambda_t = N_t/N_{t-1}$, and the mean of λ_t was calculated over all time steps.

Summary statistics

The geometric mean (±95% CI) was used as the summary statistic for mean λ_t (Morris and Doak 2002). Geometric mean λ (λ_G) was examined as a function of F for the 5-, 10-, and 20-year time frames. The rate of mortality at which λ_G was equal to one was noted as the rate of mortality at which λ_G was at replacement. The population would decline from recruitment overfishing if mortality increased beyond the rate that caused replacement.

Sensitivity analysis

A sensitivity analysis was conducted to examine which parameters influenced our model the most (Nicolson et al. 2002) and which age classes of lake trout had the largest effect on population growth rate (Caswell 2001). Commonly used analytical sensitivity and elasticity analyses based on matrix eigenvalues assume the population is at stable age distribution and are based on long-term population dynamics (Morris and Doak 2002). However, harvested populations or populations changing in abundance are not likely at stable age distribution (Fefferman and Reed 2006), and we were interested in sensitivity over the short term. Therefore, we used a conventional simulation approach to evaluate sensitivity (Cross and Beissinger 2001). The sensitivity value for each

Table 2. Regression statistics for mean length at capture through time (years; N = the number of years) from 1998 through 2007.

Age	β_0	β_1	r^2	F	P	N
4	–16 572	8.46	0.34	2.55	0.17	7
5	–2 096	1.27	0.01	0.05	0.84	8
6	–7 891	4.19	0.12	0.79	0.41	8
7	–2 240	1.40	0.09	0.52	0.50	7
8	11 203	–5.30	0.39	3.16	0.14	7
9	20 910	–10.13	0.45	2.43	0.22	5
10	49 587	–24.42	0.77	13.69	0.02	6
11	55 489	–27.34	0.55	3.62	0.15	5
12	95 960	–47.53	0.81	13.11	0.04	5

Fig. 1. Percent change in mass at length between 1998 and 2007 for lake trout in Yellowstone Lake, Yellowstone National Park. The horizontal reference line indicates no change from 1998.

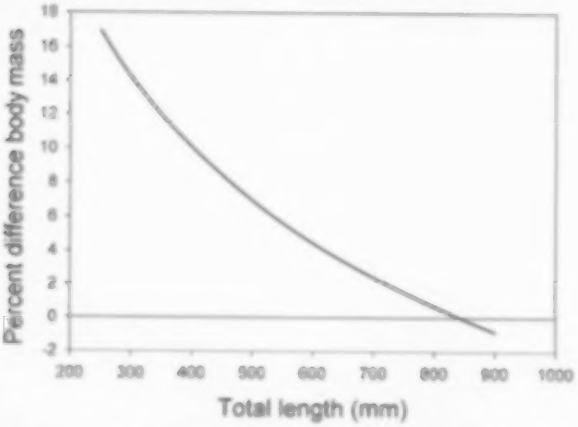
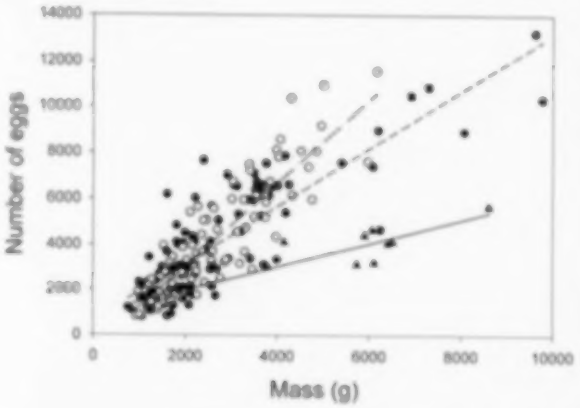


Fig. 2. Mass–fecundity regressions for 1996 (triangles), 2006 (solid circles), and 2007 (open circles) for lake trout in Yellowstone Lake, Yellowstone National Park.



vital rate was calculated as the percent reduction in λ_G at 5, 10, and 20 years given a 10% reduction in the vital rate. Sensitivities were calculated with F equal to the rate observed in 2005. For each matrix replicate in 1000 simulations, each vital rate was reduced by 10% while holding all other vital rates constant. The reduction in λ_G given each altered vital rate relative to λ_G produced by the unaltered matrix was calculated at 5, 10, and 20 time steps. The reductions in λ_G were averaged across the 1000 matrix replicates for a mean estimate of sensitivity of λ_G to each vital rate through each time frame. The sensitivity of population growth rate to total annual reproduction was estimated by summing age-specific sensitivities to fecundity across all age classes.

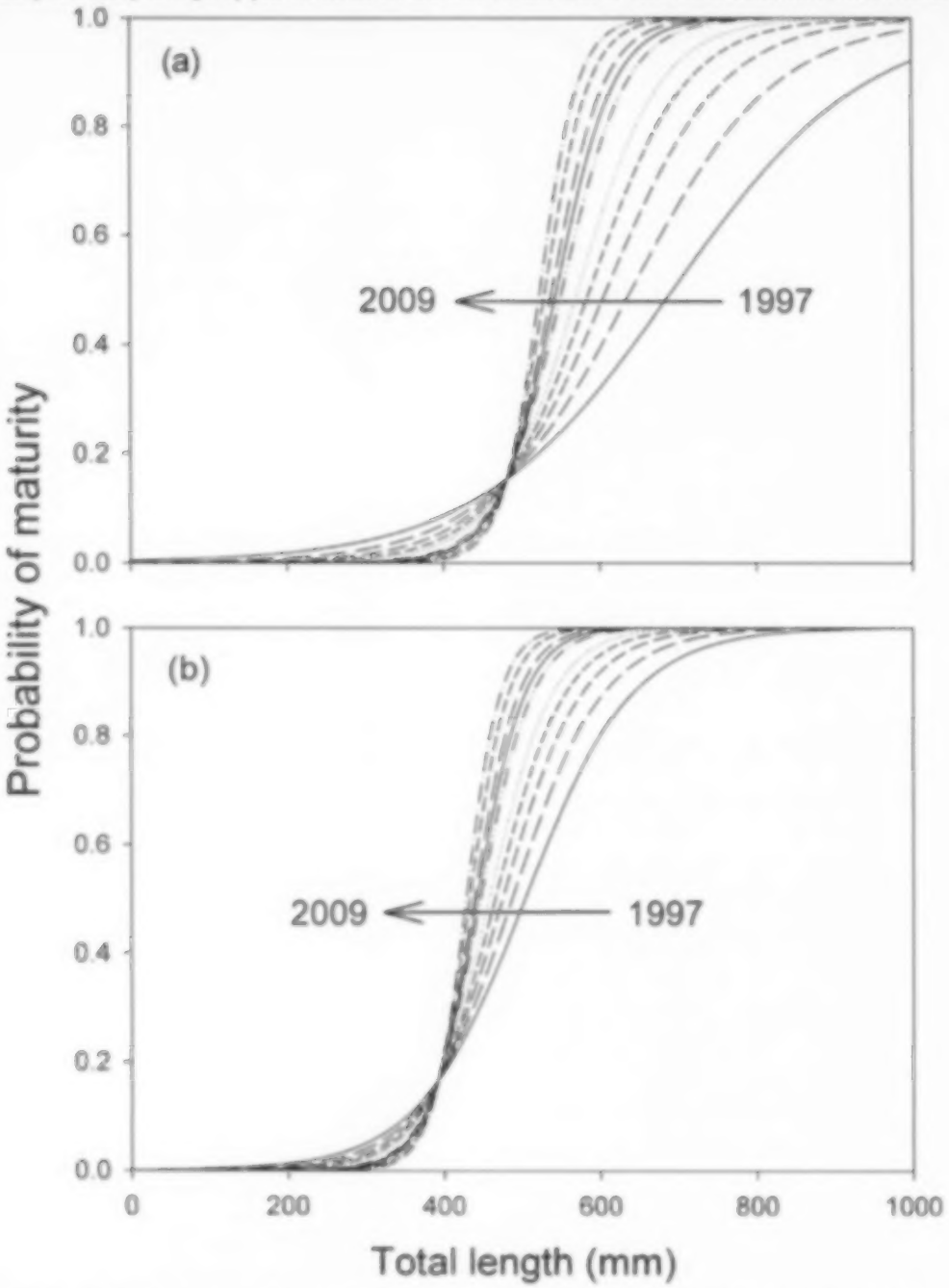
Results

Temporal analyses of population metrics yielded mixed results regarding the trend in lake trout density. Compensatory increases in growth were not detected with regressions of mean length at age through time, and mean length decreased through time for age 10 and 12 lake trout (Table 2). Mass–length regression slopes ($F_{[1,1924]} = 7.23$, $P < 0.01$) and intercepts ($F_{[1,1924]} = 71.09$, $P < 0.01$) differed between 1998 and 2007. Lake trout smaller than 840 mm weighed up to 17% more in 2007, and the difference in body mass between 1998 and 2007 decreased with increasing length (Fig. 1).

Slopes for the mass–fecundity models differed between 1996 and 2006 ($F_{[1,204]} = 6.57$, $P = 0.01$) and between 1996 and 2007 ($F_{[1,201]} = 16.75$, $P < 0.01$; Fig. 2). However, intercepts for mass–fecundity models were similar between 1996 and 2006 ($F_{[1,201]} = 0.21$, $P = 0.65$) and between 1996 and 2007 ($F_{[1,201]} = 0.056$, $P = 0.28$). Length at maturity decreased from 1997 through 2009 for males and females ($P < 0.01$). Length at probability of 50% maturity for females decreased from 694 mm in 1997 to 524 mm in 2009 (Fig. 3*a*), corresponding to a decrease in age at 50% maturity from 11.8 to 6.3 (Table 3). For males, the length at probability of 50% maturity decreased from 503 mm in 1997 to 428 mm in 2009 (Fig. 3*b*), corresponding in a decrease in age of 6.3 to 4.5 (Table 3).

Annual harvest estimated by the SCAA model closely matched the observed harvest from 2001 through 2006 (Fig. 4*a*). However, a paucity of data for recently recruited cohorts resulted in a poor fit for 2007 and 2008. Fully selected fishing mortality was likely overestimated in 2007, and variability increased through time for estimates of abundance (Figs. 4*a*–4*c*). Abundance increased from 87 042 (67 093–100 477; 95% CI) lake trout in 2001 to 305 101 (150 986–770 365; 95% CI) in 2008 (Fig. 4*c*), and mean λ calculated from annual abundance estimates was 1.21. Estimated F increased from 0.22 (0.15–0.30; 95% CI) in 2001 to 0.56 (0.38–0.76; 95% CI) in 2007 before declining to 0.18 (0.12–0.26; 95% CI) in 2008 (Fig. 4*b*). Recruitment of age-2 lake trout increased from 20 931 (12 875–30 569; 95% CI) to 129 430 (33 476–614 513; 95% CI) lake

Fig. 3. Probability of maturity at length by year for female (*a*) and male (*b*) lake trout in Yellowstone Lake, Yellowstone National Park.



trout from 2001 through 2008. The catchability coefficient, indicating the increase in F given a 1-unit increase in gill-netting effort (1 unit = 100 m of net set for 1 night), was 1.35×10^{-5} (1.03×10^{-5} to 1.69×10^{-5}; 95% CI). Selectivity was one for age-2 lake trout and gradually declined to 0.83 for age-17 lake trout (Fig. 4*d*).

Total annual mortality (A) from catch curve regressions increased from 0.24 (95% CI = 0.14–0.36) in 1997 to 0.34 (95% CI = 0.26–0.40) in 2009 (Table 4). Estimates of natural mortality were near 0.1 through the duration of the program (Table 4), with small deviations from this value caused by slightly varying VBF growth parameters (Table 3). Consequently, μ increased from 0.14 (95% CI = 0.003–0.263) in 1998 to 0.26 (95% CI = 0.19–0.32) in 2009. Estimates of Z

Table 3. Population metrics (95% confidence interval) by year for lake trout in Yellowstone Lake, Yellowstone National Park.

	Metric						
				L_{m50}		A_{m50}	
Year	t_0	K	L_∞	Female	Male	Female	Male
1997	—	—	—	694 (602–1172)	503 (456–559)	11.8 (8.6–15.0)	6.3 (5.4–7.5)
1998	0.66 (0.37–0.95)	0.18 (0.15–0.21)	807 (768–846)	641 (587–762)	487 (452–521)	9.8 (8.2–15.5)	6.0 (5.4–6.7)
1999	—	—	—	609 (572–667)	474 (448–498)	6.8 (6.0–8.2)	4.4 (4.0–4.8)
2000	–1.11 (–2.46–0.25)	0.09 (0.01–0.17)	1163 (514–1812)	588 (560–624)	465 (444–483)	6.3 (5.8–7.1)	4.3 (4.0–4.5)
2002	–0.57 (–1.26–0.12)	0.18 (0.12–0.24)	764 (678–850)	—	—	—	—
2003	–1.23 (–2.15 – –0.31)	0.10 (0.06–0.14)	1004 (853–1155)	553 (534–573)	447 (432–461)	6.5 (6.1–6.9)	4.7 (4.4–4.9)
2004	–2.73 (–4.55 – –0.91)	0.04 (0.00–0.08)	1684 (200–3170)	546 (528–565)	442 (427–457)	7.0 (6.6–7.5)	5.0 (4.7–5.3)
2005	–0.86 (–1.36 – –0.36)	0.14 (0.11–0.18)	810 (730–890)	540 (523–560)	439 (423–455)	6.6 (6.2–7.2)	4.5 (4.2–4.8)
2006	–1.91 (–2.54 – –1.28)	0.09 (0.07–0.11)	983 (850–1116)	—	—	—	—
2007	–0.47 (–0.80 – –0.13)	0.15 (0.13–0.17)	812 (765–860)	531 (514–549)	433 (416–449)	6.4 (6.0–6.9)	4.5 (4.3–4.8)
2009	—	—	—	524 (506–544)	428 (410–447)	6.3 (5.9–6.7)	4.5 (4.2–4.8)

Note: The table includes parameters t_0, K, and L_∞ from von Bertalanffy growth models (see text for definition), length (mm) at 50% maturity (L_{m50}), and age (years) at 50% maturity (A_{m50}).

Fig. 4. Statistical catch-at-age results from 1998 through 2007 for lake trout in Yellowstone Lake, Yellowstone National Park. (*a*) Observed harvest by year (connected dots) and estimated harvest (solid line). (*b*) Mean (solid line) fully selected fishing mortality (F) and 95% confidence intervals (dashed lines). (*c*) Mean lake trout abundance (solid line) and 95% confidence interval (dashed lines). (*d*) Mean selectivity (solid line) and 95% confidence intervals (dashed lines).

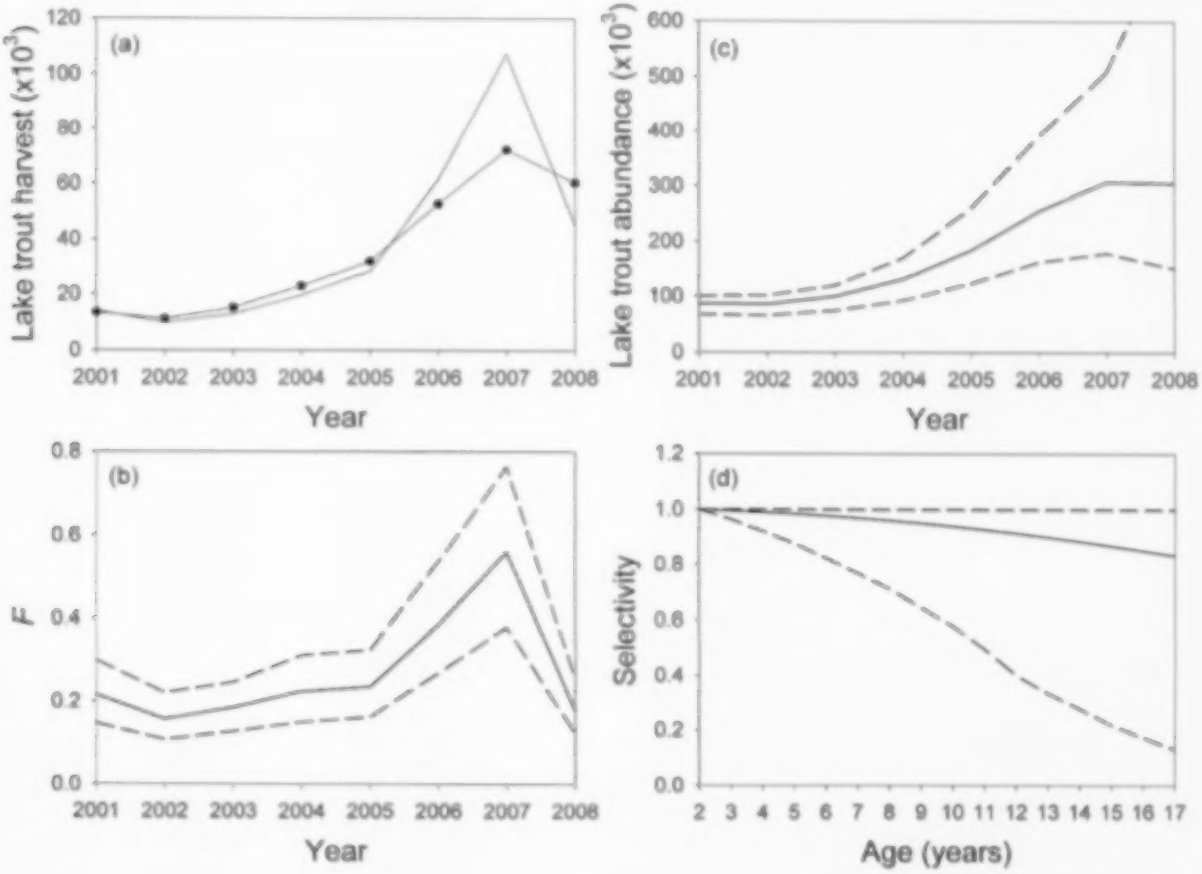


Table 4. Annual estimates of total instantaneous mortality (Z), instantaneous natural mortality (M), instantaneous fishing mortality (F), and total annual mortality (A) from catch curve regressions for lake trout in Yellowstone Lake, Yellowstone National Park.

Year	Z (SE)	M	F	A
1997	0.276 (0.049)	0.108	0.168	0.24
1998	0.262 (0.078)	0.108	0.154	0.23
2005	0.342 (0.058)	0.092	0.251	0.29
2007	0.352 (0.030)	0.095	0.257	0.30
2009	0.410 (0.048)	0.095	0.315	0.34

Note: SE, standard error.

increased through time (Table 4); however, the year effect was not significant ($F_{[1,43]} = 2.289$, $P = 0.138$). Back-calculated annual abundances of ages 2–17 lake trout increased from 49 798 (95% CI = 29 405–136 873) lake trout in 1998 to 290 038 (95% CI = 213 464–432 514) in 2009 and represent a λ of 1.2. Estimates of fishing mortality from catch curves were lesser than estimates from the SCAA, but only differed by 0.01 in 2005 (Table 4; Fig. 4*b*).

In the absence of fishing mortality, the 20-year estimate of λ_G from the matrix model was 1.29 (95% CI = 1.14–1.39). Estimates of λ_G at observed selectivity and fishing mortality ($F = 0.25$) in 2005 were 1.09 (95% CI = 0.96–1.2) for the 5-year time frame, 1.07 (95% CI = 0.94–1.19) for the 10-year time frame, and 1.07 (95% CI = 0.94–1.18) for the 20-year time frame (Fig. 5). Rates of F were more effective at reducing λ_G when maintained for longer time frames (Fig. 5). Geometric mean λ was reduced to one when F was 0.34 (A = 0.36) over a 20-year time frame and when F was 0.39 (A = 0.39) over a 5-year time frame. The upper 95% CI for λ_G was reduced to one when F was 0.52 (A = 0.46) over a 20-year time frame and when F was 0.59 (A = 0.50) over a 5-year time frame.

Population growth rate was most sensitive to S_0 (Figs. 6*a*–6*b*). A 10% reduction in S_0 caused a 1.86% reduction in λ_G over 5 years, a 1.38% reduction over 10 years, and a 1.23% reduction over 20 years. Population growth rate was also highly sensitive to survival for ages 1 through 6, and sensitivity to survival decreased after age 6 (Figs. 6*a*–6*b*). Sensitivity of λ_G to S_1–S_5 increased relative to the sensitivity to S_0 as time frames increased. Ten percent reductions in age-specific fecundities (accomplished by reducing P_i or Fec_i) never caused λ_G to decrease more than 0.25%. However, the sum of sensitivities across all P or Fec equaled the sensitivity to S_0.

Discussion

Population abundances estimated from the SCAA model and reconstructed from catch curve mortality estimates corroborated the matrix model, indicating the lake trout population was increasing despite more than a decade of removal. However, analyses of body condition, maturity, and fecundity appeared to indicate decreasing abundance. Given the increase in the lake trout population over the duration of the suppression program, apparent compensatory responses were likely caused by changing trophic dynamics. It is likely that a precipitous decline in the Yellowstone cutthroat trout population (Koel et al. 2005) increased resource availability for juvenile lake trout, which prey on similar items (Benson 1961; Ruzycki et al. 2003). Additionally, the declining Yellowstone cutthroat trout population may have contributed to the decline in body condition and growth for older lake trout because 90% of the diet for lake trout age 9 and older is Yellowstone cutthroat trout (Ruzycki et al. 2003). Future research should address temporal shifts in lake trout diets and diet overlap between Yellowstone cutthroat trout and lake trout. An alternative explanation for apparent compensatory responses is that lake trout sampled in the 1990s may have included fish from the source population, Lewis Lake (Munro et al. 2005). The lake trout population in Lewis Lake is at carrying capacity and is characterized by poor growth and body condition caused by low food availability. Lake trout were likely introduced into Yellowstone Lake as recently as the mid-1990s (Munro et al. 2005), and mass, maturity, and fecundity samples collected early in the suppression program may include those fish and reflect biological conditions in Lewis Lake rather than Yellowstone Lake.

Several population metrics indicated that lake trout have not reached carrying capacity in Yellowstone Lake. The most recent estimates of VBF growth model parameters L_∞ and ω (a measure of pre-reproductive growth calculated as the product of t_0 and K) rank in the 88th percentile when compared with 54 lake trout populations across Ontario (Shuter et al. 1998). Additionally, 50% maturity for males in Yellowstone Lake occurred at a small size and young age when compared with North American lake trout populations with varying levels of harvest (Healey 1978*a*; Trippel 1993; Sitar and He 2006). Age at 50% maturity for males in Yellowstone Lake in 2009 was similar to estimates for lake trout in Lake Michigan (Madenjian et al. 1998), where density had not recovered from collapse. Female lake trout in Yellowstone Lake matured at a similar age and smaller size than females in Lake Michigan, which matured at ages 5.5 to 7.4 and lengths of 628 to 654 mm (Madenjian et al. 1998). However, declining body condition for larger lake trout may indicate that piscivorous lake trout are approaching carrying capacity. Given ontogenetic shifts in lake trout diet (Ruzycki et al. 2003), carrying capacities likely vary for juvenile and adult lake trout and are likely to change as the forage base changes. Future population modeling may require density dependence, which is likely to reduce population growth rate (λ) at high lake trout abundance (Ferreri et al. 1995). Density-dependent reductions in growth are expected if lake trout abundance increases (Sitar and He 2006). Reductions in growth may result in later ages at maturity and reductions in fecundity (Ferreri and Taylor 1996; Madenjian et al. 1998). Conversely, reductions in lake trout density would increase the resilience of the population to reduction through shifts towards higher growth, earlier maturity, and higher fecundity rates (Zipkin et al. 2008; Johnston and Post 2009). Population metrics should be monitored into the future to determine whether density-dependent patterns are expressed.

The amount of fishing effort required to reduce mean λ to one over a 20-year time frame is 25 262 units per year, and effort must increase to 28 977 units per year to reduce λ to one over a 5-year time frame. Thus, the amount of effort expended each year should be at least the amount expended in 2007, when effort peaked at 28 500 units. Fishing effort must increase beyond these levels when considering variability in

Fig. 5. Geometric mean population growth rate (λ_G; black lines) with 95% confidence intervals (gray lines) at given levels of fully selected fishing mortality (F) over 5- (solid line), 10- (dashed line), and 20-year (dotted line) time frames for lake trout in Yellowstone Lake, Yellowstone National Park. The horizontal reference line indicates $\lambda_G = 1$ (replacement).

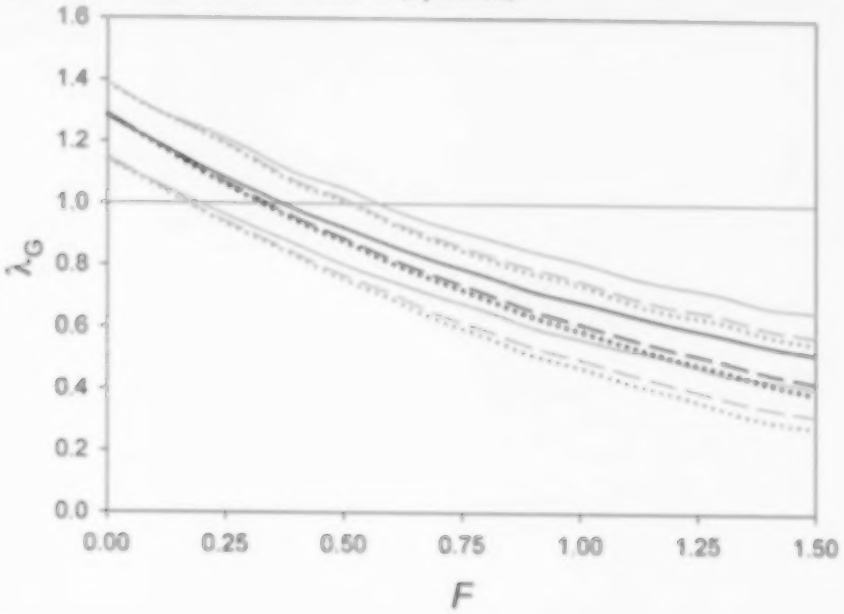
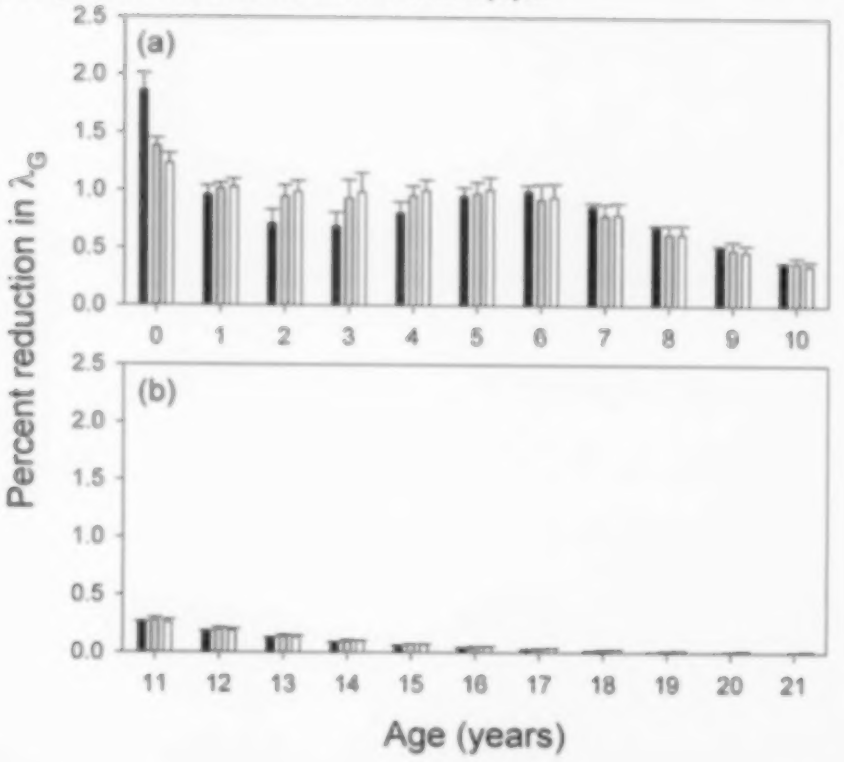


Fig. 6. Percent reduction in geometric mean population growth rate (λ_G) over 5- (black bars), 10- (gray bars), and 20-year (open bars) time frames given 10% reductions in age-specific survival for ages 0–10 (*a*) and 11–21 (*b*). Error bars delineate standard errors. Reductions in population growth rate for ages 21–30+ were less than 0.006% and are not displayed.



estimates of λ and catchability (q). For example, the lower confidence interval for q indicates that effort must be 50 315 units to reduce the upper CI of λ to one over a 20-year time frame.

Population growth rate (λ) was most sensitive to reproduction (through fecundity or S_0), indicating that increasing fishery selectivity for mature lake trout or targeting developing lake trout embryos may increase the effectiveness of lake trout suppression. Increased selectivity for mature lake trout will require increased fishing effort with large-mesh gill nets or the implementation of trap nets. A telemetry study would be useful for identifying additional spawning sites where gill net catch per unit effort is high. Although electrofishing at spawning sites produced a high catch per unit effort, depth at the majority of sites limits effectiveness (Koel et al. 2007). The current netting strategy was developed to maximize lake trout harvest while reducing bycatch of Yellowstone cutthroat trout (Koel et al. 2005). Bycatch is currently less than 0.1 Yellowstone cutthroat trout per lake trout (Koel et al. 2005); however, it may increase if mature lake trout are targeted with increased gill-netting effort, as these fish are likely to occupy depths in the upper hypolimnion in close proximity to Yellowstone cutthroat trout prey (Ruzycki et al. 2003; Dux et al. 2011). Future studies should address the effect of bycatch mortality on Yellowstone cutthroat trout population growth rate relative to predation rate and the efficiency of trap-netting versus gill-netting for lake trout suppression (Hansen et al. 2010).

The level of total annual mortality required to cause a decline in lake trout abundance in Yellowstone Lake was similar to the level of mortality required to suppress other introduced lake trout populations. A population model indicated the lake trout population in Lake Pend Oreille was likely to decline when total annual mortality (A) was between 0.45 and 0.50 (Hansen et al. 2010). Spawning potential ratio simulations for an introduced lake trout population in Lake McDonald, Glacier National Park, indicated that recruitment overfishing was likely to occur when exploitation exceeded 0.36 (A = 0.44–0.49; Dux 2005). The above studies support the assertion that lake trout populations are likely to decline when A is near 0.5 (Healey 1978*b*).

We evaluated the increase in fishing mortality required to reduce λ to 1.0 as a minimal target for management in the future; however, the goal for lake trout suppression in Yellowstone Lake is not to decrease λ to replacement, but to decrease lake trout abundance as much as possible. The few existing examples of suppression projects eliciting recovery of native fish populations indicate that reductions in predator abundance of greater than 80%–90% were required (Dudley and Matter 2000; Lepak et al. 2006). Clearly, a multiple species assessment would be useful for determining the reduction in lake trout required to elicit recovery of the Yellowstone cutthroat trout population.

For a population eradication program to succeed, the number of individuals removed must exceed the number of individuals being produced (Bomford and O'Brien 1995). Although this statement seems obvious, it highlights the importance of inflicting mortality on the population before population growth reaches the steep section of the logistic growth curve. Rapid detection and response increase the effectiveness of population eradication programs (Simberloff 2003), and the implementation of population suppression in Yellowstone Lake soon after the discovery of lake trout may have increased the probability of success. However, the lake trout suppression program also highlights the potential drawbacks of such an approach. Lake trout suppression was initiated without knowledge of population size, and harvest benchmarks that would lead to successful population decline were not determined. Consequently, the program proceeded for several years with inadequate resources, and the lake trout population continued to increase.

Eradication or suppression of introduced populations is ubiquitous because introduced species rank second to habitat destruction among threats to world-wide biodiversity (Simberloff 2001). However, the results of such projects are often not reported in the peer-reviewed literature (Simberloff 2009). Lake trout suppression has become an increasingly common management practice for the conservation of native fishes and ecosystems throughout the western USA (Martinez et al. 2009), and large-scale removals of other fish species are likely to be considered in the future (Kolar and Lodge 2002; Koehn 2004; DeGrandchamp et al. 2008). As the longest ongoing lake trout removal project, the lake trout suppression program in Yellowstone Lake provides a case study for the evaluation of a large-scale suppression effort for a non-native fish species. Lake trout suppression in Yellowstone Lake highlights the necessity for baseline data, long-term planning, and a large amount of fishing pressure to substantially reduce a non-native predator from a large, natural water body.

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References

Benson, N.G. 1961. Limnology of Yellowstone Lake in relation to the cutthroat trout. Res. Rep. 56. US Fish and Wildlife Service, Washington, D.C.

Bomford, M., and O'Brien, P. 1995. Eradication or control for vertebrate pests. Wildl. Soc. Bull. **23**(2): 249–255.

Britton, J.R., and Brazier, M. 2006. Eradicating the invasive topmouth gudgeon, *Pseudorasbora parva*, from a recreational fishery in northern England. Fish. Manag. Ecol. **13**(5): 329–335. doi:10.1111/j.1365-2400.2006.00510.x.

Cambray, J.A. 2003. Impact on indigenous species biodiversity caused by the globalization of alien recreational freshwater fisheries. Hydrobiologia, **500**(1–3): 217–230. doi:10.1023/A:1024648719995.

Caswell, H. 2001. Matrix population models: construction, analysis and interpretation, 2nd ed. Sinauer Associates, Sunderland, Mass.

Crait, J.R., and Ben-David, M. 2006. River otters in Yellowstone Lake depend on a declining cutthroat trout population. J. Mammal. **87**(3): 485–494. doi:10.1644/05-MAMM-A-205R1.1.

Cross, P.C., and Beissinger, S.R. 2001. Using logistic regression to analyze the sensitivity of PVA models: a comparison of methods

based on African wild dog models. Conserv. Biol. **15**(5): 1335–1346. doi:10.1046/j.1523-1739.2001.00031.x.

Crossman, E.J. 1995. Introduction of the lake trout (*Salvelinus namaycush*) in areas outside its native distribution: a review. J. Great Lakes Res. **21**(Suppl. 21): 17–29. doi:10.1016/S0380-1330(95)71081-4.

DeGrandchamp, K.L., Garvey, J.E., and Colombo, R.E. 2008. Movement and habitat selection by invasive Asian carp in a large river. Trans. Am. Fish. Soc. **137**(1): 45–56. doi:10.1577/T06-116.1.

Dextrase, A.J., and Mandrak, N.E. 2006. Impacts of alien invasive species on freshwater fauna at risk in Canada. Biol. Invasions, **8**(1): 13–24. doi:10.1007/s10530-005-0232-2.

Dudley, R.K., and Matter, W.J. 2000. Effects of small green sunfish (*Lepomis cyanellus*) on recruitment of Gila chub (*Gila intermedia*) in Sabino Creek, Arizona. Southwest. Nat. **45**(1): 24–29. doi:10.2307/3672548.

Dux, A.M. 2005. Distribution and population characteristics of lake trout in Lake McDonald, Glacier National Park: implications for suppression. M.Sc. thesis, Department of Ecology, Montana State University, Bozeman, Mont.

Dux, A.M., Guy, C.S., and Fredenberg, W.A. 2011. Spatiotemporal distribution and population characteristics of a nonnative lake trout population, with implications for suppression. N. Am. J. Fish. Manage. **31**(2): 187–196.

Ellis, B.K., Stanford, J.A., Goodman, D., Stafford, C.P., Gustafson, D.L., Beauchamp, D.A., Chess, D.W., Craft, J.A., Deleray, M.A., and Hansen, B.S. 2011. Long-term effects of a trophic cascade in a large lake ecosystem. Proc. Natl. Acad. Sci. U.S.A. **108**(3): 1070–1075. doi:10.1073/pnas.1013006108. PMID:21199944.

Fefferman, N.H., and Reed, J.M. 2006. A vital rate sensitivity analysis for nonstable age distributions and short-term planning. J. Wildl. Manage. **70**(3): 649–656. doi:10.2193/0022-541X(2006)70[649:AVRSAF]2.0.CO;2.

Ferreri, C.P., and Taylor, W.W. 1996. Compensation in individual growth rates and its influence on lake trout population dynamics in the Michigan waters of Lake Superior. J. Fish Biol. **49**(5): 763–777. doi:10.1111/j.1095-8649.1996.tb00077.x.

Ferreri, C.P., Taylor, W.W., and Hayes, D.B. 1995. Evaluation of age-0 survival and its effects on lake trout rehabilitation in the Michigan waters of Lake Superior. J. Great Lakes Res. **21**(Suppl. 1): 218–224. doi:10.1016/S0380-1330(95)71094-2.

Fredenberg, W. 2002. Further evidence that lake trout displace bull trout in mountain lakes. Intermt. J. Sci. **8**(3): 143–152.

Gresswell, R.E. 2009. Yellowstone cutthroat trout (*Oncorhyncus clarkii bouvieri*): a technical conservation assessment [online]. USDA Forest Service, Rocky Mountain Region. Available from www.fs.fed.us/r2/projects/scp/assessments/yellowstonecutthroattrout.pdf.

Gresswell, R.E., and Varley, J.D. 1988. Effects of a century of human influence on the cutthroat trout of Yellowstone Lake. Am. Fish. Soc. Symp. **4**: 45–52.

Gresswell, R.E., Liss, W.J., and Larson, G.L. 1994. Life-history organization of Yellowstone cutthroat trout (*Oncorhyncus clarkii bouvieri*) in Yellowstone Lake. Can. J. Fish. Aquat. Sci. **51**(S1): 298–309. doi:10.1139/f94-317.

Haddon, M. 2001. Modelling and quantitative methods in fisheries. Chapman and Hall, New York.

Hansen, M.J., Schill, D., Fredericks, J., and Dux, A. 2010. Salmonid predator–prey dynamics in Lake Pend Oreille, Idaho, USA. Hydrobiologia, **650**(1): 85–100. doi:10.1007/s10750-010-0299-3.

Healey, M.C. 1978*a*. The dynamics of exploited lake trout populations and implications for management. J. Wildl. Manage. **42**(2): 307–328. doi:10.2307/3800268.

Healey, M.C. 1978*b*. Fecundity changes in exploited populations of lake whitefish (*Coregonus clupeaformis*) and lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. **35**(7): 945–950. doi:10.1139/f78-155.

Holčík, J. 1991. Fish introductions in Europe with particular reference to its central and eastern part. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 13–23. doi:10.1139/f91-300.

Isely, J.J., and Grabowski, T.B. 2007. Age and growth. *In* Analysis and interpretation of freshwater fisheries data. *Edited by* C.S. Guy and M.L. Brown. American Fisheries Society, Bethesda, Md. pp. 187–228.

Jelks, H.L., Walsh, S.J., Burkhead, N.M., Contreras-Balderas, S., Diaz-Pardo, E., Hendrickson, D.A., Lyons, J., Mandrak, N.E., McCormick, F., Nelson, J.S., Platania, S.P., Porter, B.A., Renaud, C.B., Schmitter-Soto, J.J., Taylor, E.B., and Warren, M.L., Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries, **33**(8): 372–407.

Johnston, F.D., and Post, J.R. 2009. Density-dependent life-history compensation of an iteroparous salmonid. Ecol. Appl. **19**(2): 449–467. doi:10.1890/07-1507.1. PMID:19323202.

Kaeding, L.R., Boltz, G.D., and Carty, D.G. 1995. Lake trout discovered in Yellowstone Lake. *In* The Yellowstone Lake crisis: confronting a lake trout invasion [online]. *Edited by* J.D. Varley and P. Schullery. Report to the Director of the National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming. pp. 4–11. Available from www.nps.gov/yell/planyourvisit/upload/laketrout2.pdf.

Kaplinski, M.A. 1991. Geomorphology and geology of Yellowstone Lake, Yellowstone National Park, Wyoming. M.Sc. thesis, Department of Geology, Northern Arizona University, Flagstaff, Ariz.

Knapp, R.A., and Matthews, K.R. 1998. Eradication of nonnative fish by gill netting from a small mountain lake in California. Restor. Ecol. **6**(2): 207–213. doi:10.1111/j.1526-100X.1998.06212.x.

Koehn, J.D. 2004. Carp (*Cyprinus carpio*) as a powerful invader in Australian waterways. Freshw. Biol. **49**(7): 882–894. doi:10.1111/j.1365-2427.2004.01232.x.

Koel, T.M., Bigelow, P.E., Doepke, P.D., Ertel, B.D., and Mahony, D. L. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries, **30**(11): 10–19.

Koel, T.M., Arnold, J.L., Bigelow, P.E., Doepke, P.D., Ertel, B.D., and Ruhl, M.E. 2007. Yellowstone fisheries and aquatic sciences annual report, 2006 [online]. TCR-2007-04. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming. Available from www.nps.gov/yell/planyourvisit/fishreports.htm [updated 13 June 2011].

Kolar, C.S., and Lodge, D.M. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science, **298**(5596): 1233–1236. doi:10.1126/science.1075753. PMID:12424378.

Lepak, J.M., Kraft, C.E., and Weidel, B.C. 2006. Rapid food web recovery in response to removal of an introduced apex predator. Can. J. Fish. Aquat. Sci. **63**(3): 569–575. doi:10.1139/f05-248.

Linton, B.C., Hansen, M.J., Schram, S.T., and Sitar, S.P. 2007. Dynamics of a recovering lake trout population in eastern Wisconsin waters of Lake Superior, 1980–2001. N. Am. J. Fish. Manage. **27**(3): 940–954. doi:10.1577/M06-042.1.

Louette, G., and Declerck, S. 2006. Assessment and control of non-indigenous brown bullhead *Ameiurus nebulosus* populations using fyke nets in shallow ponds. J. Fish Biol. **68**(2): 522–531. doi:10.1111/j.0022-1112.2006.00939.x.

Madenjian, C.P., DeSorcie, T.J., and Stedman, R.M. 1998. Maturity schedules of lake trout in Lake Michigan. J. Great Lakes Res. **24**(2): 404–410. doi:10.1016/S0380-1330(98)70831-7.

Martin, N.V., and Olver, C.H. 1980. The lake charr, *Salvelinus namaycush*. *In* Charrs: salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Kluwer Boston, Inc., Hingham, Mass. pp. 205–277.

Martinez, P.J., Bigelow, P.E., Deleray, M.A., Fredenberg, W.A., Hansen, B.S., Horner, N.J., Lehr, S.K., Schneidervin, R.W., Tolentino, S.A., and Viola, A.E. 2009. Western lake trout woes. Fisheries, **34**(9): 424–442.

McDonald, M.E., and Hershey, A.E. 1989. Size structure of a lake trout (*Salvelinus namaycush*) population in an arctic lake: influence of angling and implications for fish community structure. Can. J. Fish. Aquat. Sci. **46**(12): 2153–2156. doi:10.1139/f89-266.

McIntyre, J.D. 1995. Review and assessment of possibilities for protecting the cutthroat trout of Yellowstone Lake from introduced lake trout: proceedings of a workshop and information exchange held in Gardiner, Montana, 15–17 February 1995 [online]. *In* The Yellowstone Lake crisis: confronting a lake trout invasion. *Edited by* J.D. Varley and P. Schullery. Report to the Director of the National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming. pp. 28–33. Available from www.nps.gov/yell/planyourvisit/upload/laketrout2.pdf.

Miller, R.R., Williams, J.D., and Williams, J.E. 1989. Extinctions of North American fishes during the past century. Fisheries, **14**(6): 22–38.

Miranda, L.E., and Bettoli, P.W. 2007. Mortality. *In* Analysis and interpretation of freshwater fisheries data. *Edited by* C.S. Guy and M.L. Brown. American Fisheries Society, Bethesda, Md. pp. 229–277.

Morgan, L.A., Shanks, W.C., III, Lovalvo, D.A., Johnson, S.Y., Stephenson, W.J., Pierce, K.L., Harlan, S.S., Finn, C.A., Lee, G., Webring, M., Schulze, B., Dühn, J., Sweeney, R., and Balistrieri, L. 2003. Exploration and discovery in Yellowstone Lake: results from high-resolution sonar imaging, seismic reflection profiling, and submersible studies. J. Volcanol. Geotherm. Res. **122**(3–4): 221–242. doi:10.1016/S0377-0273(02)00503-6.

Morris, W.F., and Doak, D.F. 2002. Quantitative conservation biology: theory and practice of population viability analysis. Sinauer Associates Inc., Sunderland, Mass.

Mueller, G.A. 2004. Predatory fish removal and native fish recovery in the Colorado River mainstem: What have we learned? Fisheries, **30**(9): 10–19.

Munro, A.R., McMahon, T.E., and Ruzycki, J.R. 2005. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. Can. J. Fish. Aquat. Sci. **62**(1): 79–87. doi:10.1139/f04-174.

Nicolson, C.R., Starfield, A.M., Kofinas, G.P., and Kruse, J.A. 2002. Ten heuristics for interdisciplinary modeling projects. Ecosystems, **5**(4): 376–384. doi:10.1007/s10021-001-0081-5.

Ogutu-Ohwayo, R., and Hecky, R.E. 1991. Fish introductions in Africa and some of their implications. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 8–12. doi:10.1139/f91-299.

Pauly, D. 1980. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. J. Cons. Cons. Int. Explor. Mer, **39**(2): 175–192.

Pazzia, I., Trudel, M., Ridgway, M., and Rasmussen, J.B. 2002. Influence of food web structure on the growth and bioenergetics of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. **59**(10): 1593–1605. doi:10.1139/f02-128.

Pope, K.L., and Kruse, C.G. 2007. Condition. *In* Analysis and interpretation of freshwater fisheries data. *Edited by* C.S. Guy and M.L. Brown. American Fisheries Society, Bethesda, Md. pp. 423–471.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rose, K.A., Cowan, J.H., Jr, Winemiller, K.O., Myers, R.A., and Hilborn, R. 2001. Compensatory density dependence in fish populations: importance, controversy, understanding and prognosis. Fish Fish. **2**(4): 293–327.

Ruzycki, J.R. 2004. Impact of lake trout introductions on cutthroat trout of selected western lakes of the continental United States. Ph.D. thesis, Department of Aquatic, Watershed, and Earth Resources, Utah State University, Logan, Utah.

Ruzycki, J.R., Beauchamp, D.A., and Yule, D.L. 2003. Effects of introduced lake trout on native cutthroat trout in Yellowstone Lake. Ecol. Appl. **13**(1): 23–37. doi:10.1890/1051-0761(2003)013[0023:EOILTO]2.0.CO;2.

Schullery, P., and Varley, J.D. 1995. Cutthroat trout and the Yellowstone Lake ecosystem. *In* The Yellowstone Lake crisis: confronting a lake trout invasion. *Edited by* J.D. Varley and P. Schullery. Report to the Director of the National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming. pp. 12–21. Available from www.nps.gov/yell/planyourvisit/upload/laketrout2.pdf.

Shuter, B.J., Jones, M.L., Korver, R.M., and Lester, N.P. 1998. A general, life history based model for regional management of fish stocks: the inland lake trout (*Salvelinus namaycush*) fisheries of Ontario. Can. J. Fish. Aquat. Sci. **55**(9): 2161–2177. doi:10.1139/f98-055.

Simberloff, D. 2001. Biological invasions — How are they affecting us, and what can we do about them? West. N. Am. Nat. **61**(3): 308–315.

Simberloff, D. 2003. How much population biology is needed to manage introduced species? Conserv. Biol. **17**(1): 83–92. doi:10.1046/j.1523-1739.2003.02028.x.

Simberloff, D. 2009. We can eliminate invasions or live with them. Successful management projects. Biol. Invasions, **11**(1): 149–157. doi:10.1007/s10530-008-9317-z.

Sitar, S.P., and He, J.X. 2006. Growth and maturity of hatchery and wild lean lake trout during population recovery in Michigan waters of Lake Superior. Trans. Am. Fish. Soc. **135**(4): 915–923. doi:10.1577/T05-019.1.

Sitar, S.P., Bence, J.R., Johnson, J.E., Ebener, M.P., and Taylor, W.W. 1999. Lake trout mortality and abundance in southern Lake Huron. N. Am. J. Fish. Manage. **19**(4): 881–900. doi:10.1577/1548-8675(1999)019<0881:LTMAAI>2.0.CO;2.

Stapp, P., and Hayward, G.D. 2002. Effects of an introduced piscivore on native trout: insights from a demographic model. Biol. Invasions, **4**(3): 299–316. doi:10.1023/A:1020985331815.

Stubben, C.J., and Milligan, B.G. 2007. Estimating and analyzing demographic models using the popbio package in R. J. Stat. Softw. **22**(11): 1–23.

Syslo, J.M. 2010. Demography of lake trout in relation to population suppression in Yellowstone Lake, Yellowstone National Park. M.Sc. thesis, Department of Ecology, Montana State University, Bozeman, Mont.

Theriot, E.C., Fritz, S.C., and Gresswell, R.E. 1997. Long-term limnological data from the larger lakes of Yellowstone National Park, Wyoming, U.S.A. Arct. Alp. Res. **29**(3): 304–314. doi:10.2307/1552145.

Thompson, P.D., and Rahel, F.J. 1996. Evaluation of depletion–removal electrofishing of brook trout in small Rocky Mountain streams. N. Am. J. Fish. Manage. **16**(2): 332–339. doi:10.1577/1548-8675(1996)016<0332:EODREO>2.3.CO;2.

Trippel, E.A. 1993. Relations of fecundity, maturation, and body size of lake trout, and implications for management. N. Am. J. Fish. Manage. **13**(1): 64–72. doi:10.1577/1548-8675(1993)013<0064:ROFMAB>2.3.CO;2.

Trippel, E.A. 1995. Age at maturity as a stress indicator in fisheries. Bioscience, **45**(11): 759–771. doi:10.2307/1312628.

Tronstad, L., Hall, R.O., Jr, Koel, T.M., and Gerrow, K.G. 2010. Introduced lake trout produced a four-level trophic cascade in

Yellowstone Lake. Trans. Am. Fish. Soc. **139**(5): 1536–1550. doi:10.1577/T09-151.1.

Vander Zanden, M.J., Chandra, S., Allen, B.C., Reuter, J.E., and Goldman, C.R. 2003. Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California–Nevada) Basin. Ecosystems, **6**(3): 274–288. doi:10.1007/s10021-002-0204-7.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmanek, M., and Westbrooks, R. 1997. Introduced species: a significant component of human-caused biological change. N.Z. J. Ecol. **21**(1): 1–16.

Zipkin, E.F., Sullivan, P.J., Cooch, E.G., Kraft, C.E., Shuter, B.J., and Weidel, B.C. 2008. Overcompensatory response of a smallmouth bass (*Micropterus dolomieu*) population to harvest: release from competition? Can. J. Fish. Aquat. Sci. **65**(10): 2279–2292. doi:10.1139/F08-133.

Stream salmonids as opportunistic foragers: the importance of terrestrial invertebrates along a stream-size gradient

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Abstract: Terrestrial invertebrates have been reported to be positively selected by stream salmonids. We assessed the importance of terrestrial and aquatic invertebrates to salmonid diets in 25 streams in Finland, with the hypothesis that terrestrial prey would be important in only the smallest forest streams. Several measures of prey availability were used, including proportional abundance in benthic or drift samples, compared with a trait-based approach, to predict diet composition. Across all 25 streams in autumn, blackfly and caddis larvae were the most important prey items. Terrestrial invertebrates were of moderate importance in all streams, including the smallest. Pure availability predicted diet best and provided, in most cases, a significant fit with the observed diet. In a quantitative literature review, the mean proportion of terrestrial prey in salmonid diets was 17%, being highest for the largest fish (≥15 cm). Species of the genus *Salmo* consumed significantly less terrestrials than did other salmonid genera. The proportion of terrestrial prey was highest in streams flowing through deciduous forests, but it was only weakly correlated with channel width.

Résumé : On a signalé que les salmonidés d'eau courante font une sélection positive des invertébrés terrestres. Nous évaluons l'importance des invertébrés terrestres et aquatiques dans les régimes alimentaires deS salmonidés dans 25 cours d'eau de Finlande avec comme hypothèse que les proies terrestres ne devraient être significatives que dans les plus petits cours d'eau forestiers. Nous avons utilisé plusieurs mesures de disponibilité des proies, en particulier l'importance proportionnelle dans les échantillons de benthos ou de dérive, par comparaison aux méthodes basées sur les traits, pour prédire la composition du régime alimentaire. Sur l'ensemble des 25 cours d'eau en automne, les larves de mouches noires et de phryganes constituent les proies individuelles les plus importantes. Les invertébrés terrestres sont d'importance moyenne dans tous les cours d'eau, y compris les plus petits. La simple disponibilité est la meilleure variable prédictive du régime et permet, dans la plupart des cas, un ajustement significatif avec le régime observé. Dans une revue quantitative de la littérature, la proportion moyenne de proies terrestres dans les régimes de salmonidés est de 17 % et elle est maximale chez les poissons les plus grands (≥15 cm). Les espèces du genre *Salmo* consomment significativement moins de proies terrestres que les autres genres de salmonidés. La proportion de proies terrestres est maximale dans les cours d'eau qui traversent des forêts décidues; elle n'est que faiblement corrélée à la largeur du chenal.

[Traduit par la Rédaction]

Introduction

Dietary composition and prey selectivity of stream-dwelling salmonids have been addressed in numerous studies that have typically focused on one fish species in a particular stream or stream system, often at just one sampling time (Cada et al. 1987; Miyasaka et al. 2003; Baxter et al. 2004). Generalizations of the determinants of salmonid diets have proved difficult, owing to, for example, spatially and temporally variable taxonomic composition of prey assemblages in streams. Thus far, the most systematic efforts for a generalization have been produced by Rader (1997) and De Crespin De Billy and Usseglio-Polatera (2002) in an attempt to explain and predict salmonid diets based on the relative vulnerability of benthic invertebrates to fish predation. Their models were thus based on invertebrate traits known, or assumed, to be of importance to prey selection by salmonids.

Salmonid fishes are traditionally considered visually hunting, day-active, sit-and-wait predators that feed on invertebrates drifting in the water column (Elliott 1973; Fraser and Metcalfe 1997). They select foraging sites to minimize energy costs and maximize the availability of drifting prey per unit time (Nakano and Kaeriyama 1995; Young et al. 1997). They may also attack epibenthic prey, particularly in darkness

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(Nakano and Furukawa-Tanaka 1994; Kreivi et al. 1999), but this strategy is considered of moderate importance. However, when drift is reduced experimentally, epibenthic foraging may become the predominant foraging mode (Nislow et al. 1998). The choice of a foraging strategy is also contingent on the social status of a fish, with less dominant (and usually smaller) fish feeding disproportionately from the benthos (Nakano et al. 1999). This may cause strong intra- and inter-population variation in salmonid diets (e.g., Bridcut and Giller 1995), further complicating any generalizations based simply on prey availability in the drift. Salmonids are also size-selective predators, feeding disproportionately on the largest and (or) most conspicuous prey items (Allan 1981; Meissner and Muotka 2006). One aspect of this behavior is selective feeding on terrestrial invertebrates that fall on the water surface. While floating downstream, these prey are highly conspicuous and vulnerable to attacks by drift-feeding fish (Nakano et al. 1999). On the other hand, prey availability in streams is often limited (Johnson et al. 2006), potentially driving fish towards omnivorous feeding. Indeed, several studies have shown that salmonids are opportunistic foragers, with diets closely reflecting the composition of benthic and (or) drift samples (Allan 1981; Bres 1986; Esteban and Marchetti 2004). Benthic invertebrate densities in boreal streams decline to their annual minimum during summer (Poff and Huryn 1998), whereas availability and activity of riparian invertebrates often peaks at the same time, potentially causing fish to focus strongly on terrestrial prey during summer. Such a seasonally variable importance of terrestrial invertebrates to salmonid diets has recently been detected in many fish populations (e.g., Kawaguchi and Nakano 2001; Nakano and Murakami 2001).

The importance of terrestrial prey to salmonids may also depend on stream width, with greatest importance in small headwater streams. There are at least two potential, not mutually exclusive, mechanisms causing such a pattern. First, in small, heavily shaded streams, the riparian canopy often extends over the entire channel, thus delivering terrestrial prey over the entire stream surface. Food webs in such streams, as also in lakes with undeveloped riparian forests, are heavily subsidized by inputs of terrestrial prey, although this riparian subsidy may exhibit strong seasonality (Nakano and Murakami 2001; Francis and Schindler 2009). As the river gets wider, terrestrial invertebrates will fall on a smaller proportion of the stream surface, contributing relatively less to the prey base. Second, even if the input of terrestrial prey to salmonid food base remains constant with increasing channel width, relative terrestrial contribution to diet may still decline if benthic invertebrate production increases downstream. Also, not only the mere presence of riparian vegetation but, even more importantly, the riparian vegetation type affects the availability of terrestrial prey, with deciduous trees providing more terrestrial subsidies to stream fish than coniferous trees (Wipfli 1997; Allan et al. 2003).

We analyzed prey availability for and use by brown trout (*Salmo trutta*) in 25 streams, spanning a size gradient from small (2–3 m wide) headwater streams to fifth-order rivers (up to 80 m wide) in central and northern Finland. Most of our data represent late summer – early autumn conditions (late August to mid-September), but we also include corresponding early summer (early June) data for seven streams. These two seasons represent the periods of the fastest growth of brown trout in boreal forest streams (e.g., Korsu et al. 2009). Further, we evaluated the ability of Rader's (1997) model to predict the diet of brown trout in our study streams, adjusting the model to site-specific conditions by estimating prey abundance in each stream. We specifically assessed the importance of terrestrial invertebrates to trout diets across a stream-size gradient. Finally, to place our results in a wider perspective, we extracted data from literature on the use of terrestrial prey by salmonid fishes in relation to stream size and other explanatory variables (fish size and genus; riparian vegetation type).

Materials and methods

Study streams and field protocol

We divided the 25 study streams into three size groups: small (mean channel width < 3.5 m, $n = 10$), medium-sized (4–12 m, $n = 8$), and large (>20 m, $n = 7$) streams (Table 1), roughly corresponding to first- to second-order, third-order, and fourth- to fifth-order streams, respectively. One of the medium-sized streams and four of the large streams were lake outlets. The sampling site in each stream consisted mainly of stony riffles, covering an area of 100–1000 m^2. Water quality was good to excellent (see Table 1), and the stream channels were hydromorphologically unmodified or only slightly modified. Riparian forests were mainly mixed, with 2–10 m high deciduous trees (alder, *Alnus* spp.; birch, *Betula* spp.; and bird cherry *Prunus padus*) and willows (*Salix* spp.) and 5–20 m high coniferous trees (spruce, *Picea abies*; pine, *Pinus sylvestris*). In early autumn (August to September), we sampled all 25 streams for both trout and benthic organisms; seven of the streams (six small and one medium-sized) were also sampled for drift. To assess seasonal variability in trout diets, we sampled some of the streams (five small and two medium-sized) also in the early summer (early June). Brown trout reproduce naturally in all of the study streams, and although stocking is practiced in some of the streams, about 95% of fish were wild-born (based on fin condition; J. Syrjänen, personal observation). Trout populations in the small streams are generally resident, but in the medium-sized and large streams they are partly lake-migrating (Saraniemi et al. 2008; Syrjänen and Valkeajärvi 2010). We collected trout of mainly 11–22 cm in total length (ages 1 to 3) with a backpack electrofishing gear (GeOmega FA3, pulsed direct current 50 Hz, 350–700 V). We then anaesthetized the fish with carbon dioxide and flushed their stomachs with 3–4 dL of water. This method is nondetrimental to fish, removing more than 95% of stomach contents (Light et al. 1983; Sotiropoulos et al. 2006). Number of fish collected per stream varied between 14 and 48. After full recovery from anesthesia, we released the fish to their site of capture. Kreivi et al. (1999) showed that in late summer the feeding of age-1 trout in a subarctic Finnish river peaked at 0600 to 0900 h. Therefore, to ascertain that gut contents would represent recent feeding, we conducted all fish sampling between 1000 and 1400 h.

We measured prey availability in benthos by collecting kick net samples (0.30 mm mesh size) from the same reach just before fish sampling. When collecting a sample, substrate in front of the net was disturbed to the depth of 5–10 cm for 30 s. We measured prey availability in drift using polyvinyl chloride (PVC) tubes fitted with a 1 m long net (11 cm diameter, net mesh size 0.30 mm, 5–10 nets per

Table 1. Location, size, selected water chemistry characteristics, and density of brown trout (individuals per 100 m^2) in the 25 study streams in Finland.

Stream	Latitude (N)	Longitude (E)	Mean channel width (m)	Stream order	pH	Total P ($\mu g \cdot L^{-1}$)	Trout density
Small							
Hirvasjoki	62°30′	25°42′	3.8	2	7.0	13	5
Kiertojoki	61°59′	26°03′	1.7	2	6.2	5	36
Köhniönpuro	62°15′	25°40′	1.6	2	7.0	12	11
Särkioja	65°40′	28°11′	3.0	2	6.0	NA	40
Ukonoja	65°44′	27°45′	2.6	2	5.8	NA	60
Kostonlamminoja	65°36′	28°02′	1.5	2	7.2	11	62
Virsuoja	65°22′	27°39′	2.6	2	6.4	18	68
Jefestimänjoki	66°12′	29°32′	3.0	3	NA	NA	NA
Koivupuro	65°46′	27°50′	2.1	1	NA	NA	NA
Raatepuro	66°15′	29°40′	2.8	2	7.3	9	5
Medium							
Arvajanjoki	61°41′	25°10′	12	3	6.6	4	145
Könkköjoki	62°14′	25°16′	7.0	3	6.4	19	30
Rutajoki	61°59′	25°59′	8.0	3	6.8	9	35
Saajoki	61°59′	25°24′	4.3	3	6.1	16	24
Loukusanjoki	65°35′	27°57′	6.7	3	6.7	12	12
Ohtaoja	65°36′	28°03′	6.0	3	7.5	22	NA
Porontimanjoki	66°12′	29°21′	7.0	3	7.3	9	4
Räätpysoja	65°40′	28°21′	7.0	3	6.7	16	NA
Large							
Huopanankoski	63°33′	25°02′	20	4	6.9	12	20
Muuramenjoki	62°08′	25°41′	19	4	6.9	9	28
Ripatinkoski	61°36′	26°40′	20	5	7.0	5	72
Siikakoski	62°37′	26°21′	80	4	7.0	6	56
Simunankoski	62°23′	26°11′	42	5	7.1	7	54
Kitkajoki	66°15′	29°28′	40	4	7.3	7	15
Kuusinkijoki	66°17′	29°54′	45	4	7.2	17	45

Note: NA, no data available.

stream). The drift samplers operated for 3–4 h before fish sampling, just above the area to be electrofished. To calculate drift densities, we measured current velocity at the mouth of each net at the beginning and end of a sampling period. At each site where drift was collected, we positioned some of the nets 3–5 cm above the substratum, thus sampling subsurface drift, while others were positioned so that they intersected water surface, thus sampling mainly surface drift (aerial stages of aquatic insects and terrestrial invertebrates). We collected three to five kick net and drift samples at each site. We identified invertebrates mainly to genus level and counted their numbers in the laboratory. Counts of individuals were used in all analyses. We pooled the numbers of prey used by trout across all individuals in each stream, as well as numbers of prey available across all samples in each stream. Thus, each stream produced one data point.

Prey selectivity by trout

We grouped prey taxa into eight functionally and (or) taxonomically similar groups that dominated (by numbers) in the benthic–drift samples and trout diet: baetid mayfly nymphs (Baetidae), nonpredatory stonefly nymphs (mainly families Leuctridae and Nemouridae), case-bearing caddis larvae (Trichoptera), net-spinning caddis larvae (Trichoptera), midge larvae (Chironomidae), blackfly larvae (Simuliidae), adult stages of aquatic insects, and terrestrial invertebrates. These eight groups comprised 79% of prey use (by numbers) in early summer and 75% in early autumn. We compared prey availability with prey consumption using Chesson's (1983) preference index (α) calculated for each of the eight prey groups in each stream:

$$\alpha = \left(\frac{r_i}{n_i}\right)\left(\sum_{j=1}^{m}\frac{r_j}{n_j}\right)^{-1}$$

where r is the proportion of prey type i in the diet, n is the proportion of prey type i in a benthic–drift sample, and m is the number of prey categories included in the analysis. The index was calculated separately for benthic and drift samples (when available). We tested the index values for each stream size category (small, medium, large) by one-sample t test against neutral selectivity, estimated as $1/m$, where m is the number of prey categories included. To reduce the risk of type I error, we set the alpha level a priori at $\alpha = 0.01$.

Correlations between prey use and availability: test of Rader's model

Rader (1997) classified aquatic invertebrates based on 11

traits that determine their availability to salmonid fishes: benthic exposure, body size, and nine categories describing drift propensity (intentional drift, habitat, flow exposure, mobility, drag index, drift distance, emergence behavior, ovipositing behavior, and diel activity). Rader (1997) assigned each prey type to each of these categories, using scores from 0 to 9, and then summed the subtotal group scores. For final group scores, subtotal scores were weighted by multipliers depending on the abundance of each prey (see next section for details). Taxonomically, Rader's prey groups were insect genera or families and larger clusters for other aquatic invertebrates. To test the applicability of Rader's model to our data, we used exactly the same prey groups and scores as Rader (1997) for all prey taxa that occurred in both our data and in his classification. Four of the prey taxa observed in this study (leeches, Hirudinea; the cased caddis family Goeridae and genus *Sericostoma*, the isopod *Asellus aquaticus*) were not included in Rader's classification. We therefore asked four experienced benthic invertebrate ecologists to provide their independent scores for these taxa for each of the eight traits used by us (see below) and then used a consensus scoring in data analysis (see online Supplemental Table S1[1]).

We used Spearman rank correlations to test for the correspondence between trout diet and several measures of prey availability to examine whether a trait-based approach would predict diet better than assuming that consumption was proportional to prey availability in benthic or drift samples: (*i*) diet composition (proportional abundance of each prey type) vs. Rader's (1997) scores; (*ii*) diet vs. Rader's scores combined with site-specific abundance in benthic samples; (*iii*) diet vs. relative abundance in benthos; (*iv*) diet vs. Rader's scores combined with site-specific abundance in drift samples; and (*v*) diet vs. relative abundance in drift. However, we focused on only those eight of Rader's original traits that characterize aquatic life forms–stages, excluding three (emergence behavior, ovipositing behavior, diel activity) that characterize adult stages of aquatic insects. We used Rader's original abundance categories based on regional abundance (obtained from literature) in (*i*) and Rader's multipliers (0.5, 1.0, 1.2, 1.5) for scores based on abundance in (*i*), (*ii*), and (*iv*). We estimated average subtotal scores (ASS) for the eight traits included and only for the taxonomic categories that occurred in our data. We used ASS in (*i*), (*ii*), and (*iv*) to determine the multiplier values. We included site-specific abundance (*ii*, *iv*) to mimic Rader's generalized abundance classification. Thus, we categorized each prey type into one of three abundance classes (abundant, common, rare) based on their abundance in stream benthos (*ii*) or drift (*iv*). A prey category was determined abundant if its proportion in availability samples was more than 20%, common if it was 3%–20%, and rare if it was less than 3%. Following Rader's (1997) final (total) scoring, we multiplied subtotal scores by 0.5 (rare taxa), 1.0 (common and abundant taxa with scores less than mean ASS), 1.2 (common taxa with scores exceeding mean ASS), or 1.5 (abundant taxa with scores exceeding mean ASS). To avoid the strong impact on correlations by a large number of zeros, as well as the many ties caused by rare taxa (Esteban and Marchetti 2004), we only included taxa that comprised more than 0.1% of the mean use and availability across all streams in each season. The total number of taxonomic categories thus included was 18 (early summer) or 30 (early autumn). We considered correlations significant at $\alpha < 0.01$.

We used nonmetric multidimensional scaling (NMDS) ordination to summarize and compare visually patterns in trout diet vs. benthic samples based on their taxonomic composition. We ran NMDS in an autopilot mode, letting the program choose the best ordination solution (i.e., solution with the lowest stress value) from 100 separate runs of real data using PC-ORD software (McCune and Mefford 1999). We then used Procrustes analysis with ProTest to test for pairwise concordance between the two ordination configurations. Procrustean fitting was based on the least-squares criterion, which minimizes the sum of the squared residuals (m^2) between two ordinations, using the m^2 statistic as the measure of association. Low values of m^2 indicate strong concordance between availability (benthic samples) and use (stomach samples) across study streams. ProTest extends Procrustes analysis by providing a permutation test for the significance of the Procrustean fit (Peres-Neto and Jackson 2001). ProTest randomly permutes original observations in one matrix so that each site can be assigned any of the values attributed to other sites. The m^2 statistic is recalculated for each permutation, and the proportion of the statistic smaller than or equal to the observed value provides the significance level of the test. We used the program ProTest (Jackson 1995) with 9999 permutations. Because of sample size limitations, we only conducted these analyses for the early autumn samples. We used multi-response permutation procedure (MRPP) to analyse differences in community composition among stream-size classes, separately for dietary and benthic samples. MRPP is a nonparametric method for testing differences in assemblage structure between a priori defined groups. The null hypothesis of no difference among groups was tested by a Monte Carlo procedure with 1000 permutations.

The importance of terrestrial invertebrates to salmonid diets: a quantitative review

To quantify the general importance of terrestrial invertebrates to the diet of stream salmonids, we performed a literature survey on studies published between 1973 and 2009 using Biological Abstracts and Google Scholar. We used various combinations of the following key words: trout, salmon, charr, food, feeding, diet, foraging, prey, predation, and terrestrial. We obtained additional papers by examining reference lists of the original articles and by including our own data (this study) to the final database. We considered individual streams as data points (replicates). For each study, we recorded the percentage of terrestrial (aquatic adult insects not included) invertebrates in the diet of salmonids, as well as several explanatory variables, if available. As we initially suspected that terrestrial prey would be important for salmonids mainly in small streams, we included channel width as a primary explanatory variable in our analysis. In addition, we used fish size class, salmonid genus, and riparian vegetation type as additional explanatory variables. We divided channel width into four size classes: <3, 3–8, 8–20, >20 m. Fish total length was divided into three size classes: <80, 80–149,

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2011-118.

and ≥150 mm. Salmonids were classified in three genera: *Salmo*, *Oncorhynchus*, and *Salvelinus*. Riparian vegetation type was classified into four categories: deciduous forest, coniferous forest, mixed forest, and open canopy (streams bordered by bush, grass, or open bank). If a study included information on several fish size classes, fish genera, and (or) riparian vegetation type, we included each as a separate data point. We only included summer months (June to August in the Northern Hemisphere; December to February in the Southern Hemisphere) in our data. In data analysis, we set channel width (four levels), vegetation type (four levels), fish size (three levels), and fish genus (three levels) as factorial variables. We analysed the statistical significance of each factor to the proportion of terrestrials in salmonid diets using generalized least squares model (GLS) (gls function in nlme library) available in the free software R 2.11.1 (R Development Core Team 2010). Because of data limitations, we only included two-way interactions in the maximal model and used manual model simplification (partial F tests) to obtain the minimal adequate model. We set the significance level at $\alpha = 0.05$ and arcsine-transformed the proportion of terrestrial items used by fish to better meet the assumption of normality. To deal with data heteroscedasticity, VarIdent function, allowing groups to have different variance (Pinheiro and Bates 2000), was fitted in the model.

Results

Prey use and selectivity by trout

The total number of fish sampled was 297 in June and 878 in August–September. The proportion of fish with empty stomachs was 1% in June (range 0% to 9%) and 7% in early autumn (range 0% to 31%). Nine brown trout had ingested other fish (mainly sculpin).

Across all 25 streams (ten small, eight medium-sized, and seven large streams) in early autumn, blackfly larvae and case-bearing and net-spinning caddis larvae (in order of importance) were the most important prey groups in trout diet (Fig. 1), whereas larval stages of baetid mayflies, net-spinning caddis, and chironomid midges dominated the benthos. Drift was generally dominated by baetid, chironomid, and blackfly larvae, and to a lesser degree, and in small streams only, by terrestrial invertebrates (Supplemental Table S2[1]). Differences in diet related to stream size were obvious, however. In small streams, diet was dominated by terrestrial invertebrates, blackfly larvae, and case-bearing caddis larvae. Only chironomid larvae deviated from random selection in both benthic and drift samples, being significantly under-represented in the diet of trout ($P < 0.001$). Five of the prey groups present in drift samples were significantly under-represented in the diet, whereas none were significantly over-represented (Fig. 1*a*). In medium-sized streams, only cased caddis larvae were significantly over-represented ($P = 0.004$), whereas baetid mayfly, stonefly, and chironomid larvae were under-represented in the diet (all $P < 0.01$) (Fig. 1*c*). In large streams, trout used mostly caddis larvae and blackfly larvae. Baetids and midge larvae were significantly under-represented in trout diet (both $P < 0.01$) (Fig. 1*e*). In early summer (five small and two medium-sized streams), the most important prey group in trout diet, as well as in benthic and drift samples, was larval blackflies. In small streams, no prey type was over-represented, but chironomid and blackfly larvae in both drift and benthos, and terrestrial invertebrates in drift, were significantly under-represented (all $P < 0.005$) (Fig. 1*b*). In medium-sized streams, low sample size prevented statistical comparisons, but trout seemed to use chironomid larvae and terrestrial invertebrates less than expected in these streams (Fig. 1*d*).

In the NMDS ordination plots, a stream-size gradient was evident, for both benthos and trout diet (Fig. 2). The m^2 statistic (0.713; $P = 0.012$) indicated that the two ordinations were strongly concordant (i.e., trout gut contents resembled closely benthic communities in their taxonomic composition). According to MRPP, the difference in taxonomic composition between stream size classes was significant for the diet samples ($A = 0.052$, $P = 0.011$), whereas it was somewhat weaker for the benthic samples ($A = 0.042$, $P = 0.061$).

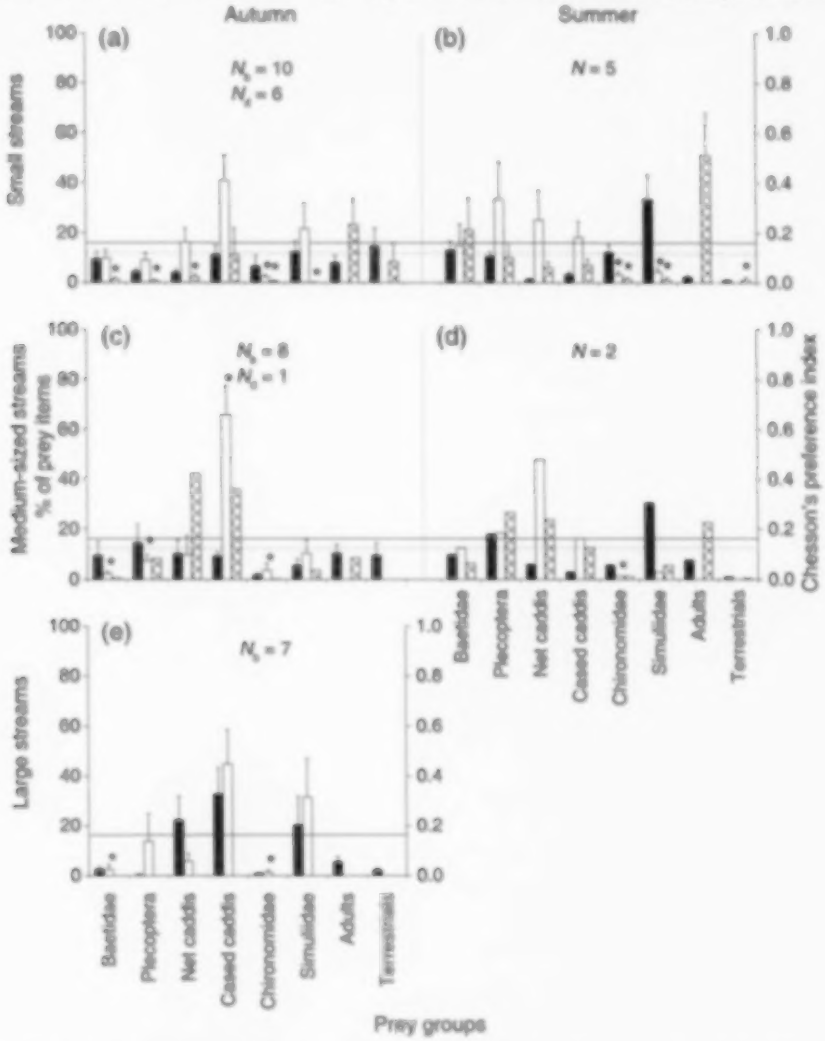
The percentage of terrestrial items in trout guts was 1.1% (±1 standard error, SE = 0.2%) in small and 1.1% (±0.1%) in medium-sized streams in early summer. In early autumn, the corresponding figures were 14.8% (±7.4%) for small, 9.6% (±4.9%) for medium-sized, and 2.2% (±0.9%) for large streams. In the early autumn data, the proportion of terrestrial invertebrates in brown trout diet across the 25 streams was weakly, though nonsignificantly, negatively related to channel width ($r^2 = 0.142$, $P = 0.063$). The most important groups of terrestrial prey in trout diet were ants (Formicidae, mainly non-winged), aphids (Aphididae), spiders (Araneae), insect caterpillars (mainly Lepidoptera), and a combined group of sawflies, wasps, and bees (Hymenoptera other than ants).

Correlations between prey use and availability

Rader's (1997) model with eight invertebrate traits predicted the prey use by brown trout moderately weakly in the early summer (Table 2). For small streams, the average Spearman correlation across all five streams in the June samples was 0.446, one of the five correlations being significant ($P = 0.002$). The correspondence with the observed diet was consistently lower when Rader's scores were combined with site-specific abundance in either benthic (mean $r_s = 0.409$; number of significant correlations = 1/5) or drift (0.348 and 1/5, respectively) samples. Pure availability produced the best predictions of trout diet (benthic samples: mean $r_s = 0.761$, number of significant correlations = 5/5; drift samples: 0.671 and 4/5, respectively). Number of mid-sized streams sampled in June was only two, but correlations showed the same pattern as for small streams, with pure proportional abundance providing the best fit with trout diet (Table 2).

Rader's model was even less successful in predicting the early autumn prey use, average correlations between trout diet and unmodified Rader's scores, or scores combined with site-specific abundances, ranging between 0.150 and 0.374. Less than one-third of the correlations were significant (Table 2). By contrast, pure availability provided a significant fit with the observed diet in 67%–100% of cases, average correlations ranging from 0.512 (drift abundances in a mid-sized stream) to 0.654 (drift abundances in a small stream). Furthermore, closely similar results were obtained irrespective of how availability was defined, reflecting the fact that the taxonomic composition of benthos and drift correlated significantly ($P < 0.01$) on both seasons, with 64% of all stream-specific correlations being significant (Table 2).

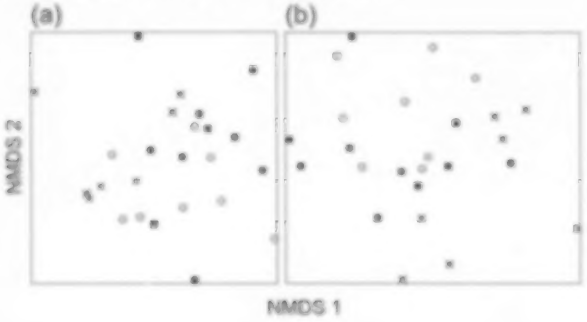
Fig. 1. Trout mean use (% of all prey items; black bars) of and mean selectivity vs. benthos (open bars) and selectivity vs. drift (hatched bars) for the eight most important prey types in small (*a*, *b*), medium-sized (*c*, *d*), and large (*e*) streams in early autumn and early summer. Sample sizes for each comparison (selectivity in relation to benthic samples, N_b; selectivity in relation to drift samples, N_d) are indicated in each figure. For diet samples, each stream provided one data point, and the number of streams included is the same as for N_b. Error bars indicate one standard error of the mean. Solid line shows the level of random feeding (or neutral selectivity) in relation to proportional availability in benthos; dotted line shows the relation to drift. Asterisks indicate significant difference ($P < 0.01$; one-sample *t* test) from neutral selectivity.



Terrestrial invertebrates in salmonid diets: a quantitative review

We found 41 articles (Supplemental Appendix S1[1]) where the proportion of terrestrial food consumed by stream salmonids was given (or could be calculated based on information presented); overall, these studies produced 95 data points. Adding our own data (17 data points from this paper and 10 from K. Korsu, unpublished data), the sample size increased to 122, representing stream-living populations of three salmonid genera: *Salmo*, *Oncorhynchus*, and *Salvelinus* from four continents: Europe ($n = 51$ studies), North America (43), Japan (12), and New Zealand (16). The overall mean proportion of terrestrial prey in salmonid diets was 17%. All other explanatory variables but stream size (see Fig. 3*a*) differed significantly among the levels included (Table 3). Thus, fish size was strongly related to the proportion of terrestrial prey in salmonid diets, with the percentage increasing from the mean of 12% in the smallest (<80 mm) to 25% in the largest (≥150 mm) size class (Fig. 3*b*). Representatives of the genus *Salmo* consumed the least terrestrials (mean of 9%), whereas the percentage was considerably higher in *Oncorhynchus* (27%) and *Salvelinus* (23%) (Fig. 3*c*). The percentage of terrestrial prey was highest in streams flowing through deciduous forests (30%), intermediate in mixed forests (13%), and lowest in coniferous forests and non-woody vegetation (11% both) (Fig. 3*d*). Only one interaction was significant

Fig. 2. NMDS (nonmetric multidimensional scaling) ordination diagrams for the benthic samples (*a*) (final stress for two-dimensional solution: 0.170) and trout stomach samples (*b*) (stress: 0.151) in the 25 streams sampled in early autumn. Squares are for small streams, open circles for medium-sized streams, and crosses for large streams. For stream size definitions, see text.



(vegetation type × salmonid genus), reflecting the fact that a great majority of studies including *Salmo* species were conducted in strongly forested (mainly mixed forests) watersheds. Also, the interaction between riparian vegetation type and stream width bordered at significance ($F_{[1,118]} = 2.93$, $P = 0.089$), possibly indicating that stream width was only important in relatively small streams with mainly deciduous canopies.

The effect of stream width was obscured by the surprisingly low importance of terrestrial items in the smallest streams (i.e., <3 m wide). Thus, the percentage of terrestrials in fish diet was only 14%, on average, in the smallest streams, while it was much higher (21%) in the second-smallest stream size class (3 to 8 m), and 19% even in streams of 8 to 20 m in width (Fig. 3*a*). Focusing on streams wider than 3 m, a distinct size gradient was established, with the proportion of terrestrial prey in fish diet decreasing with increasing stream size ($F_{[1,85]} = 45.43$, $P < 0.001$). However, in linear regression, this gradient bordered at significant for the largest fish only (Fig. 4), as also indicated by a significant channel width × fish size interaction (for data excluding the smallest streams) ($F_{[1,85]} = 12.48$, $P < 0.001$); that is, the largest fish included proportionally more terrestrial invertebrates in their diet in relatively small streams, while no such pattern was evident for smaller fish.

Discussion

Prey availability for and prey use and selectivity by brown trout

The use of different prey types by brown trout largely paralleled prey availability, resulting in a diet dominated by blackfly larvae, case-bearing caddis larvae, and terrestrial invertebrates in small streams grading to a diet dominated by case-bearing and net-spinning caddis larvae in large streams. Seasonally, a gradient from blackfly-dominated diet in early summer to terrestrial invertebrates and case-bearing caddis larvae in early autumn was detected. This pattern of prey use closely tracks seasonal variation of benthic communities in boreal streams (Malmqvist 1994; Erkinaro and Erkinaro 1998). Overall, brown trout was a highly opportunistic forager, feeding on most prey types present, roughly proportional to their availability. Prey selectivity, however, differed slightly from prey use; trout showed a weak overall selectivity for caddis larvae, a rather strong under-representation of chironomid larvae, and a weaker under-representation of baetid mayfly and, in some cases, blackfly larvae. These patterns of selectivity likely reflect size-selective feeding by brown trout, a behavior repeatedly reported for salmonids (e.g., Allan 1981; Bechara et al. 1993; Meissner and Muotka 2006). Case-bearing and net-spinning caddis larvae are among the largest prey types present in boreal streams, and in experimental studies, trout have significantly reduced densities of these prey, while chironomids may even benefit from the presence of trout (Englund et al. 1999; Meissner and Muotka 2006). As chironomid larvae are usually among the smallest prey types available, they may be of little importance to fish longer than 10 cm, but may still be of high value to age-0 fish, particularly as these start exogenous feeding after emergence from the gravel (Keeley and Grant 1997; Steingrímsson and Gíslason 2002). Larval blackflies are also relatively small, but because of their high availability in early summer and preference for exposed microhabitats (Meissner et al. 2009), they may be a seasonally important component of salmonid diets.

The observation that trout behave as an opportunistic forager was also reflected in the fact that pure prey availability, estimated from either benthic or drift samples, produced very high correlations with trout diets. Benthic biomass in our study area declines to a minimum in mid- to late summer (Haapala et al. 2001), and prey availability, particularly that of large invertebrate prey, is much higher in early summer (Kreivi et al. 1999; see also Utz and Hartman 2006); yet, correlations between use and availability were even higher in early summer. It thus appears that very simple behavioral rules (e.g., focus on most abundant prey, prefer the largest prey) regulate trout foraging, and more sophisticated models including behavioural traits of invertebrates may not be needed in most cases. Furthermore, we feel that any model explaining trout diets must include terrestrial invertebrates, while many of the traits included in Rader's (1997) or De Crespin De Billy and Usseglio-Polatera's (2002) models are not readily adjustable to terrestrial prey (or adult stages of aquatic insects). Also, these models are largely based on the latest instars of aquatic insects, while ontogenetic changes in behavioural traits may lead to wide variation in the predation risk encountered by a prey species during its aquatic life.

Interestingly, correlations between trout diet vs. benthos and diet vs. drift were about the same order. Thus, trout seemed to sample these partly overlapping prey communities quite extensively. Trout frequently ingested prey that rarely enter the drift (e.g., cased caddis larvae). Our study thus supports the view that brown trout feed directly off the bottom more often than is commonly believed. Most previous studies suggesting this have been based on evidence from just one or a few salmonid populations in lakes (Tremblay and Magnan 1991; Venne and Magnan 1995) or streams (e.g., Angradi and Griffith 1990; Kreivi et al. 1999; Amundsen et al. 2000), and our data with 25 streams provide thus far the strongest observational evidence of the potential importance of epibenthic feeding in genus *Salmo*. In several studies, salmonids have been shown to feed on benthos when drift is depleted (Nislow et al. 1998; Nakano et al. 1999). However,

Table 2. Spearman rank correlation coefficients (mean and range) for five streams in the early summer (June) and 25 streams in early autumn (August–September).

Season	Stream size	*n*	Type of correlation	Mean	Range	No. of significant correlations
Summer	Small	5	R	0.446	0.175–0.679	1
		5	RB	0.409	0.109–0.752	1
		5	B	0.761	0.712–0.828	5
		5	RD	0.348	0.080–0.603	1
		5	D	0.671	0.432–0.805	4
		5	BD	0.643	0.564–0.779	4
Autumn	Small	10	R	0.233	0.054–0.518	1
		10	RB	0.370	0.205–0.675	1
		10	B	0.625	0.458–0.892	8
		6	RD	0.324	0.166–0.637	2
		6	D	0.654	0.400–0.889	4
		6	BD	0.508	0.110–0.825	3
	Medium	8	R	0.150	–0.048–0.276	0
		8	RB	0.231	–0.044–0.391	0
		8	B	0.556	0.368–0.676	7
	Large	7	R	0.200	0.044–0.604	1
		7	RB	0.374	0.201–0.599	2
		7	B	0.606	0.496–0.782	7

Note: R = prey use vs. Rader's scores for eight invertebrate traits and abundance (based on original regional abundance categories); RB = prey use vs. Rader's scores for the eight traits, combined with site-specific abundance in benthos; B = prey use vs. pure availability (proportional abundance) in benthos; RD = prey use vs. Rader's scores for the eight traits combined with site-specific abundance in drift; D = prey use vs. pure availability in drift; and BD = prey abundance in benthos vs. prey abundance in drift. Correlations are based on proportional abundances of prey taxa in benthic, drift, or diet samples. Correlations are considered significant at $\alpha < 0.01$.

these observations were made in daylight, and as drift-feeding salmonids are visual foragers, they are likely to be severely impaired at low light levels (Fraser and Metcalfe 1997; Young et al. 1997). Case-bearing caddis, as well as many terrestrial invertebrates, are relatively large and conspicuous prey and may therefore be detected even at very low light levels, using perhaps cues other than vision (e.g., hydrodynamic cues). It is known that salmonids forage preferentially at night, but feed also during the daylight hours to satisfy their daily energy needs (Orpwood et al. 2006). We find it hard to find any other explanation but epibenthic feeding for the frequent selectivity for such rarely drifting prey as case-bearing caddis larvae (see also Forrester et al. 1994). At present, however, this hypothesis is based on correlation and must be corroborated by experiments and direct behavioral observations of individual fish, at all times of day and on different seasons representing different prey availabilities.

Terrestrial invertebrates in salmonid diets

Our finding of a relatively low amount of, and lack of selectivity for, terrestrial invertebrates in the diet of brown trout led us to suspect that the current doctrine about the great importance of terrestrial prey to salmonids may be biased, reflecting the fact that most studies showing this have been conducted in small streams with heavily forested canopies. Terrestrial invertebrate prey are often larger than aquatic prey (see Romaniszyn et al. 2007; Rosenfeld and Raeburn 2009). This, however, is not always the case; for example, Meissner and Muotka (2006) showed that while terrestrial prey were, on average, among the largest prey types available for brown trout in a Finnish forest stream, they ranged a large size gradient and were generally about the same size or even smaller than the largest benthic invertebrates (i.e., predatory invertebrates, particularly perlodid stonefly larvae and rhyacophilid caddis larvae). Therefore, we do not believe that the fact that we measured numbers of prey instead of biomass caused any serious bias to our results. Clearly, however, the quantitative literature survey showed that our results may be more of an exception than a general pattern, as the meta-analysis distinctly corroborated the importance of terrestrial invertebrates to salmonids. Not surprisingly, terrestrial prey increased in importance with fish size and in streams with mainly deciduous riparian canopy. A more interesting observation was that the use of terrestrial prey was only weakly correlated with channel width. This was contrary to our expectation, particularly as the use of terrestrial prey peaked at channels of 3–8 m, not in the narrowest ones. This seems to suggest that the effective range of terrestrial prey input from the riparian canopy may be wider than commonly believed, and the riparian subsidy of stream food webs may not, therefore, be restricted to headwater systems but may prove to be important also in larger rivers.

Another interesting observation stemming from our quantitative review was that the use of terrestrial invertebrates was size-dependent, as the largest fish included proportionally more terrestrial invertebrates in their diet than did smaller fish. Such a relationship has also been documented in several previous studies (e.g., Montori et al. 2006; Dineen et al. 2007). This pattern can be caused by at least two mechanisms: (*i*) salmonids defend feeding territories, with larger fish usually occupying the best drift-feeding positions; then, if terrestrial prey are the largest prey type available in the

Fig. 3. Mean (±1 standard error, SE) proportion of terrestrial invertebrates in salmonid diets in relation to channel width (*a*), fish size (*b*), salmonid genus (*c*), and riparian vegetation type (*d*). Numbers above each bar indicate the sample size (number of streams included in each category).

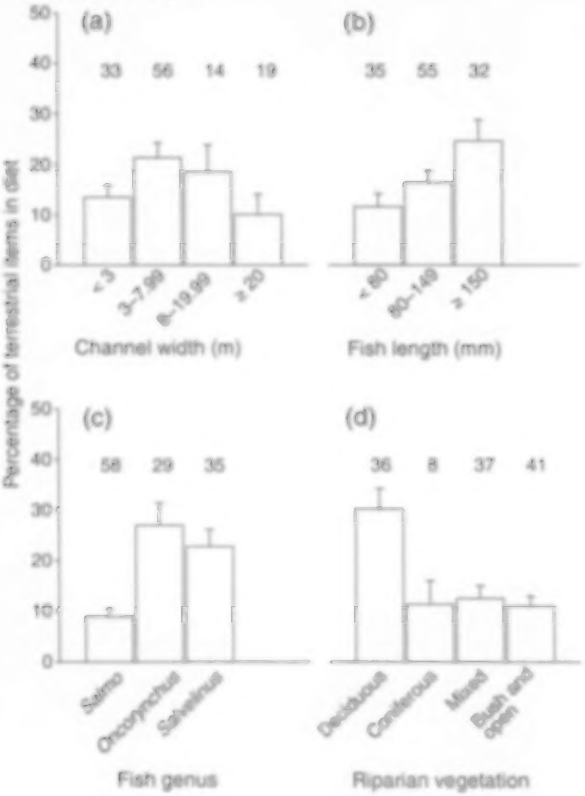


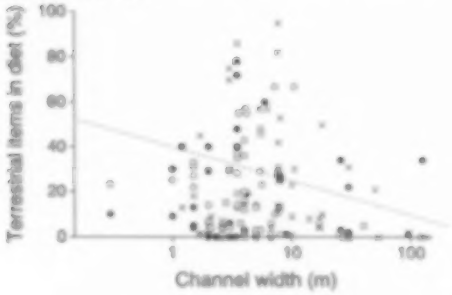
Table 3. Generalized least squares model for the proportion of terrestrial invertebrates in the diet of salmonid fishes based on a quantitative literature survey.

Source of variation	$F_{[df]}$	P
Vegetation	$11.48_{[1,120]}$	<0.001
Channel width	$0.05_{[1,120]}$	0.820
Fish length	$6.65_{[1,120]}$	0.011
Salmonid genus	$18.48_{[1,120]}$	<0.001
Vegetation × genus	$10.09_{[1,118]}$	0.002
Vegetation × channel width	$2.93_{[1,119]}$	0.089
Vegetation × fish length	$0.01_{[1,119]}$	0.987
Channel width × fish length	$0.27_{[1,119]}$	0.606
Channel width × genus	$2.63_{[1,118]}$	0.107
Fish length × genus	$1.24_{[1,118]}$	0.267

Note: All variables were included in the model as factorial, and Varident function, allowing heterogeneity of variances, was fitted in the model. All main effects and two-way interactions are reported.

drift, the large, dominant fish will disproportionately ingest these profitable prey items. Alternatively, (*ii*) size-specific differences in the use of terrestrial prey may be explained by gape limitation, with smaller fish being unable to ingest the largest invertebrate prey (Gowan 2007). Both of these hypotheses are based on terrestrial prey being, on average, larger than aquatic prey. Thus, one explanation for the relatively low proportion of terrestrial invertebrates in trout diet in our field survey might have been that terrestrial and aquatic prey did not differ appreciably in size and that brown trout in our streams seemed to be feeding relatively intensively on epibenthic invertebrates.

Fig. 4. Relationship between channel width and the proportion of terrestrial invertebrates in salmonid diets, separately for each fish length class (<80, 80–149, ≥150 mm). Solid circles are for fish shorter than 80 mm, open circles for fish of 80–149 mm, and crosses for fish equal or longer than 150 mm. The overall relationship (all fish size classes combined) was nonsignificant, and linear regression bordered significance only for the largest fish length class ($r^2 = 0.078$, $P = 0.055$).



The most surprising finding of our review was that salmonid genera differed vastly, with *Salmo* ingesting terrestrial items much less frequently than did the other two genera. This may reflect different habitat preferences and (or) foraging strategies in different species. For example, Korsu et al. (2010) observed age-1 brook trout (*Salvelinus fontinalis*) to select primarily slow-flowing pool sections, while brown trout of the same age preferred riffles. Such species-specific differences in habitat use may cause different salmonid species to encounter partly differing prey assemblages. Terrestrial invertebrates drifting on the water surface in pools should be highly visible for fish, but the more turbulent flow in riffles may render surface-drifting (i.e., terrestrial) invertebrates less easily detectable. Unfortunately, studies comparing the foraging behavior and prey selectivity of *Salmo* spp. with those of other salmonids are surprisingly rare. In one of the few exceptions, Mookerji et al. (2004) showed that while Atlantic salmon (*Salmo salar*) preferred aquatic insects, brook trout fed mainly upon terrestrial invertebrates.

Allan et al. (2003) proposed that deciduous trees are of better food quality for herbivores than are coniferous trees, and thus deciduous riparian forests should deliver more invertebrates to streams. Our findings, and those of Romero et al. (2005), support this assertion, as the proportion of terrestrial prey in trout diets was higher in streams draining deciduous forests. Similarly, the use of terrestrial prey by fish was much higher in lakes with intact riparian forest than in developed, open-shore lakes in North America (Francis and Schindler 2009).

As most studies of salmonid diets are conducted in later summer or autumn, they may miss the most important season for parr growth in boreal streams, that is, spring and early summer. The total food intake of parr is often highest in early summer (Allan 1981; Kreivi et al. 1999), presumably because

of the high availability of aquatic prey then. This was also indirectly reflected in our study, where the contribution of riparian invertebrates to the early summer diet of brown trout was minimal. Thus, studies focusing on mid- to late summer diets alone may provide a biased estimate of the importance of terrestrial prey to salmonid diets. Indeed, Nakano and Murakami (2001) reported the proportion of terrestrial prey to be 23%–57% of the total annual energy intake of four salmonid species (no *Salmo* species included) in a Japanese forest stream. Although riparian invertebrates were not strongly preferred by brown trout in our study, they can still be of critical importance for the overwintering success of trout. For example, terrestrial invertebrates may be a key component of trout diet in late September, when many riparian invertebrates are still active but large-sized benthic prey are scarce. Overall, the proportional importance of terrestrial invertebrates compared with aquatic prey in trout diet seems to follow a similar seasonal pattern in boreal streams draining conifer-dominated forests and more southern, deciduous forest streams (Romero et al. 2005, Romaniszyn et al. 2007), being low in winter and spring, low to moderate in summer, and relatively high in late summer and autumn.

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References

Allan, J.D. 1981. Determinants of diet of brook trout (*Salvelinus fontinalis*) in a mountain stream. Can. J. Fish. Aquat. Sci. **38**(2): 184–192. doi:10.1139/f81-024.

Allan, J.D., Wipfli, M.S., Caouette, J.P., Prussian, A., and Rodgers, J. 2003. Influence of streamside vegetation on inputs of terrestrial invertebrates to salmonid food webs. Can. J. Fish. Aquat. Sci. **60**(3): 309–320. doi:10.1139/f03-019.

Amundsen, P.-A., Gabler, H., Herfindal, T., and Riise, L.S. 2000. Feeding chronology of Atlantic salmon parr in subarctic rivers: consistence of nocturnal feeding. J. Fish Biol. **56**(3): 676–686. doi:10.1111/j.1095-8649.2000.tb00765.x.

Angradi, T.R., and Griffith, J.S. 1990. Diel feeding chronology and diet selection of rainbow trout (*Oncorhynchus mykiss*) in the Henry's Fork of the Snake River, Idaho. Can. J. Fish. Aquat. Sci. **47**(1): 199–209. doi:10.1139/f90-022.

Baxter, C.V., Fausch, K.D., Murakami, M., and Chapman, P.L. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. Ecology, **85**(10): 2656–2663. doi:10.1890/04-138.

Bechara, J.A., Moreau, G., and Hare, L. 1993. The impact of brook trout (*Salvelinus fontinalis*) on an experimental stream benthic community: the role of spatial and size refugia. J. Anim. Ecol. **62**(3): 451–464. doi:10.2307/5194.

Bres, M. 1986. A new look at optimal foraging behavior: rule of thumb in the rainbow trout. J. Fish Biol. **29**(Suppl. sA): 25–36. doi:10.1111/j.1095-8649.1986.tb04996.x.

Bridcut, E.E., and Giller, P.S. 1995. Diet variability and foraging strategies in brown trout (*Salmo trutta*): an analysis from subpopulations to individuals. Can. J. Fish. Aquat. Sci. **52**(12): 2543–2552. doi:10.1139/f95-845.

Cada, G.F., Loar, J.M., and Sale, M.J. 1987. Evidence of food limitation of rainbow and brown trout in southern Appalachian soft-water streams. Trans. Am. Fish. Soc. **116**(5): 692–702. doi:10.1577/1548-8659(1987)116<692:EOFLOR>2.0.CO;2.

Chesson, J. 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology, **64**(5): 1297–1304. doi:10.2307/1937838.

De Crespin De Billy, V., and Usseglio-Polatera, P. 2002. Traits of brown trout prey in relation to habitat characteristics and benthic invertebrate communities. J. Fish Biol. **60**(3): 687–714. doi:10.1111/j.1095-8649.2002.tb01694.x.

Dineen, G., Harrison, S.S.C., and Giller, P.S. 2007. Diet partitioning in sympatric Atlantic salmon and brown trout in streams with contrasting riparian vegetation. J. Fish Biol. **71**(1): 17–38. doi:10.1111/j.1095-8649.2007.01441.x.

Elliott, J.M. 1973. The food of brown and rainbow trout (*Salmo trutta* and *S. gairdneri*) in relation to the abundance of drifting invertebrates in a mountain stream. Oecologia, **12**(4): 329–347. doi:10.1007/BF00345047.

Englund, G., Sarnelle, O., and Cooper, S.D. 1999. The importance of data-selection criteria in meta-analyses of stream predation experiments. Ecology, **80**(4): 1132–1141. doi:10.1890/0012-9658(1999)080[1132:TIODSC]2.0.CO;2.

Erkinaro, H., and Erkinaro, J. 1998. Feeding of Atlantic salmon, *Salmo salar* L., parr in the subarctic River Teno and three tributaries in northernmost Finland. Ecol. Freshw. Fish, **7**(1): 13–24. doi:10.1111/j.1600-0633.1998.tb00168.x.

Esteban, E.M., and Marchetti, M.P. 2004. What's on the menu? Evaluating a food availability model with young-of-the-year Chinook salmon in the feather river, California. Trans. Am. Fish. Soc. **133**(3): 777–788. doi:10.1577/T03-115.1.

Forrester, G.E., Chace, J.G., and McCarthy, W. 1994. Diel and density-related changes in food consumption and prey selection by brook charr in a New Hampshire stream. Environ. Biol. Fishes, **39**(3): 301–311. doi:10.1007/BF00005131.

Francis, T.B., and Schindler, D.E. 2009. Shoreline urbanization reduces terrestrial insect subsidies to fishes in North American lakes. Oikos, **118**(12): 1872–1882. doi:10.1111/j.1600-0706.2009.17723.x.

Fraser, N.H.C., and Metcalfe, N.B. 1997. The costs of becoming nocturnal: feeding efficiency in relation to light intensity in juvenile Atlantic salmon. Funct. Ecol. **11**(3): 385–391. doi:10.1046/j.1365-2435.1997.00098.x.

Gowan, C. 2007. Short term cues used by foraging trout in a California stream. Environ. Biol. Fishes, **78**(4): 317–331. doi:10.1007/s10641-006-9099-z.

Haapala, A., Muotka, T., and Markkola, A.-M. 2001. Breakdown and macroinvertebrate and fungal colonization of alder, birch and willow leaves in a boreal forest stream. J. N. Am. Benthol. Soc. **20**(3): 395–407. doi:10.2307/1468037.

Jackson, D.A. 1995. PROTEST: a PROcrustean Randomization TEST of community environment concordance. Ecoscience, **2**: 297–303.

Johnson, R.L., Blumenshine, S.C., and Coghlan, S.M. 2006. A bioenergetic analysis of factors limiting brown trout growth in an Ozark tailwater river. Environ. Biol. Fishes, **77**(2): 121–132. doi:10.1007/s10641-006-9059-7.

Kawaguchi, Y., and Nakano, S. 2001. Contribution of terrestrial invertebrates to the annual resource budget for salmonids in forest and grassland reaches of a headwater stream. Freshw. Biol. **46**(3): 303–316. doi:10.1046/j.1365-2427.2001.00667.x.

Keeley, E.R., and Grant, J.W. 1997. Allometry of diet selectivity in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **54**(8): 1894–1902. doi:10.1139/cjfas-54-8-1894.

Korsu, K., Huusko, A., and Muotka, T. 2009. Does the introduced brook trout (*Salvelinus fontinalis*) affect growth of the native

brown trout (*Salmo trutta*)? Naturwissenschaften, **96**(3): 347–353. doi:10.1007/s00114-008-0482-9. PMID:19050841.

Korva, K., Huusko, A., and Muotka, T. 2010. Invasion of north European stream by brook trout: hostile takeover or preadapted habitat niche segregation? Biol. Invasions, **12**(5): 1363–1375. doi:10.1007/s10530-009-9553-x.

Kreivi, P., Muotka, T., Huusko, A., Mäki-Petäys, A., Huhta, A., and Meissner, K. 1999. Diel feeding periodicity, daily ration and prey selectivity in juvenile brown trout in a subarctic river. J. Fish Biol. **55**(3): 553–571. doi:10.1111/j.1095-8649.1999.tb00699.x.

Light, R.M., Adler, P.H., and Arnold, D.E. 1983. Evaluation of gastric lavage for stomach analyses. N. Am. J. Fish. Manage. **3**(1): 81–85. doi:10.1577/1548-8659(1983)3<81:EOGLFS>2.0.CO;2.

Malmqvist, B. 1994. Preimaginal blackflies (Diptera: Simuliidae) and their predators in a central Scandinavian lake outlet stream. Ann. Zool. Fenn. **31**: 245–255.

McCune, B., and Mefford, M.J. 1999. PC-ORD. Multivariate analysis of ecological data. Version 4.0. MjM Software, Gleneden Beach, Ore., USA.

Meissner, K., and Muotka, T. 2006. The role of trout in stream food webs: integrating evidence from field surveys and experiments. J. Anim. Ecol. **75**(2): 421–433. doi:10.1111/j.1365-2656.2006.01063.x. PMID:16637995.

Meissner, K., Juntunen, A., Malmqvist, B., and Muotka, T. 2009. Predator–prey interactions in a variable environment: responses of a caddis larva and its blackfly prey to variations in stream flow [online]. Ann. Zool. Fenn. **46**: 193–204. Available from http://www.sekj.org/PDF/anz46-free/anz46-193.pdf [accessed 2 June 2011].

Miyasaka, H., Nakano, S., and Furukawa-Tanaka, T. 2003. Food habit divergence between white-spotted charr and masu salmon in Japanese mountain streams: circumstantial evidence for competition. Limnology, **4**(1): 1–10. doi:10.1007/s10201-002-0088-4.

Montori, A., Tierno De Figueroa, J.M., and Santos, X. 2006. The diet of the brown trout *Salmo trutta* (L.) during the reproductive period: size-related and sexual effects. Int. Rev. Hydrobiol. **91**(5): 438–450. doi:10.1002/iroh.200510899.

Mookerji, N., Weng, Z., and Mazumder, A. 2004. Food partitioning between coexisting Atlantic salmon and brook trout in the Sainte-Marguerite River ecosystem, Quebec. J. Fish Biol. **64**(3): 680–694. doi:10.1111/j.1095-8649.2004.00333.x.

Nakano, S., and Furukawa-Tanaka, T. 1994. Intra- and interspecific dominance hierarchies and variation in foraging tactics of two species of stream-dwelling chars. Ecol. Res. **9**(1): 9–20. doi:10.1007/BF02347237.

Nakano, S., and Kaeriyama, M. 1995. Summer microhabitat use and diet of four sympatric stream-dwelling salmonids in a Kamchatkan stream. Fish. Sci. **61**: 926–930.

Nakano, S., and Murakami, M. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proc. Natl. Acad. Sci. U.S.A. **98**(1): 166–170. doi:10.1073/pnas.98.1.166. PMID:11136253.

Nakano, S., Fausch, K.D., and Kitano, S. 1999. Flexible niche partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charr. J. Anim. Ecol. **68**(6): 1079–1092. doi:10.1046/j.1365-2656.1999.00355.x.

Nislow, K.H., Folt, C., and Seandel, M. 1998. Food and foraging behavior in relation to microhabitat use and survival of age-0 Atlantic salmon. Can. J. Fish. Aquat. Sci. **55**(1): 116–127. doi:10.1139/f97-222.

Orpwood, J.E., Griffiths, S.W., and Armstrong, J.D. 2006. Effects of food availability on temporal activity patterns and growth of Atlantic salmon. J. Anim. Ecol. **75**(3): 677–685. doi:10.1111/j.1365-2656.2006.01088.x. PMID:16689950.

Peres-Neto, P.R., and Jackson, D.A. 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. Oecologia, **129**(2): 169–178. doi:10.1007/s004420100720.

Pinheiro, J.C., and Bates, D.M. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, New York.

Poff, N.L., and Huryn, A.D. 1998. Multi-scale determinants of secondary production in Atlantic salmon (*Salmo salar*) streams. Can. J. Fish. Aquat. Sci. **55**(S1): 201–217. doi:10.1139/d98-013.

R Development Core Team. 2010. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3–900051–08–9. Available from http://www.r-project.org [accessed 2 June 2011].

Rader, R.B. 1997. A functional classification of the drift: traits that influence invertebrate availability to salmonids. Can. J. Fish. Aquat. Sci. **54**(6): 1211–1234. doi:10.1139/f97-025.

Romaniszyn, E.D., Hutchens, J.J., and Wallace, J.B. 2007. Aquatic and terrestrial invertebrate drift in southern Appalachian mountain streams: implications for trout food resources. Freshw. Biol. **52**(1): 1–11. doi:10.1111/j.1365-2427.2006.01657.x.

Romero, N., Gresswell, R.E., and Li, J.L. 2005. Changing patterns in coastal cutthroat trout (*Oncorhynchus clarki clarki*) diet and prey in a gradient of deciduous canopies. Can. J. Fish. Aquat. Sci. **62**(8): 1797–1807. doi:10.1139/f05-099.

Rosenfeld, J.S., and Raeburn, E. 2009. Effects of habitat and internal prey subsidies on juvenile coho salmon growth: implications for stream productive capacity. Ecol. Freshw. Fish, **18**(4): 572–584. doi:10.1111/j.1600-0633.2009.00372.x.

Saraniemi, M., Huusko, A., and Tahkola, H. 2008. Spawning migration and habitat use of adfluvial brown trout, *Salmo trutta*, in a strongly seasonal boreal river. Boreal Env. Res. **13**: 121–132.

Sotiropoulos, J.C., Nislow, K.H., and Ross, M.R. 2006. Brook trout, *Salvelinus fontinalis*, microhabitat selection and diet under low summer stream flows. Fish. Manag. Ecol. **13**(3): 149–155. doi:10.1111/j.1365-2400.2006.00487.x.

Steingrímsson, S.Ó., and Gíslason, G.M. 2002. Body size, diet and growth of landlocked brown trout, *Salmo trutta*, in the subarctic River Laxa, North-East Iceland. Environ. Biol. Fishes, **63**(4): 417–426. doi:10.1023/A:1014976612970.

Syrjänen, J., and Valkeajärvi, P. 2010. Gillnet fishing drives lake-migrating brown trout to near extinction in the Lake Päijänne region, Finland. Fish. Manag. Ecol. **17**(2): 199–208. doi:10.1111/j.1365-2400.2010.00738.x.

Tremblay, S., and Magnan, P. 1991. Interactions between two distantly related species, brook trout (*Salvelinus fontinalis*) and white sucker (*Catostomus commersoni*). Can. J. Fish. Aquat. Sci. **48**(5): 857–867. doi:10.1139/f91-102.

Utz, R.M., and Hartman, K.J. 2006. Temporal and spatial variation in the energy intake of brook trout (*Salvelinus fontinalis*) population in an Appalachian watershed. Can. J. Fish. Aquat. Sci. **63**(12): 2675–2686. doi:10.1139/f06-152.

Venne, H., and Magnan, P. 1995. The impact of intra- and interspecific interactions on young-of-the-year brook charr, in temperate lakes. J. Fish Biol. **46**(4): 669–686. doi:10.1111/j.1095-8649.1995.tb01103.x.

Wipfli, M.S. 1997. Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. Can. J. Fish. Aquat. Sci. **54**(6): 1259–1269. doi:10.1139/f97-034.

Young, M.K., Rader, R.B., and Belish, T.A. 1997. Influence of macroinvertebrate drift and light on the activity and movement of Colorado River cutthroat trout. Trans. Am. Fish. Soc. **126**(3): 428–437. doi:10.1577/1548-8659(1997)126<0428:IOMDAL>2.3.CO;2.

Differences in antipredator behaviour between wild and hatchery-reared juvenile Atlantic salmon (*Salmo salar*) under seminatural conditions

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Abstract: Captive rearing may elicit experiential or genetically based changes to salmonid antipredator behaviour, which may reduce survival of hatchery-reared fishes when they are released into the wild. Nevertheless, few studies have compared the behaviour between local wild-caught and local hatchery-reared fish (two generations or less), and none have done so under natural conditions. We conducted a seminatural field study comparing the antipredator behaviour of wild-caught, F1 (offspring of wild-caught adults) with that of F2 (second generation) hatchery-reared young-of-the-year Atlantic salmon (*Salmo salar*, from the same source population) in response to standardized chemical alarm cues. Wild-caught salmon exhibited the strongest antipredator response to a standardized threat (conspecific alarm cues), while F2 salmon exhibited the weakest response. F1 salmon were intermediate in their behavioural response. The observed differences between wild-caught and F1 salmon suggests that differential experience may play a significant role in predator avoidance behaviours. Furthermore, the observed differences between F1 and F2 salmon suggests that even one full generation of hatchery rearing may be sufficient to select for maladaptive responses to predators under natural conditions. Given the controversy regarding the effectiveness of hatchery programs for conservation use, the results of this study suggest that minimizing hatchery time may reduce the behavioural differences between wild and hatchery-reared fishes.

Résumé : L'élevage en captivité peut provoquer des changements dus à l'expérience ou à la génétique dans le comportement anti-prédateur des salmonidés, ce qui peut réduire la survie des poissons de pisciculture lorsqu'ils sont libérés en nature. Néanmoins, peu d'études ont comparé les comportements entre des poissons capturés localement en nature et des poissons élevés localement en pisciculture (deux générations ou moins) et aucune ne l'a fait dans des conditions naturelles. Nous avons mené une étude de terrain en conditions semi-naturelles pour comparer le comportement anti-prédateur en présence de signaux chimiques standards d'alerte chez des jeunes saumons atlantiques (*Salmo salar*, de la même population d'origine) de l'année, soit des poissons capturés en nature, soit des poissons F1 (rejetons de poissons adultes capturés en nature) et soit des poissons F2 (deuxième génération) élevés en pisciculture. Les saumons capturés en nature affichent la réaction anti-prédateur la plus marquée à une menace standardisée (signaux d'alerte conspécifiques), alors que les saumons F2 montrent la réaction la plus faible. Les saumons F1 ont une réaction comportementale intermédiaire. Les différences observées entre les saumons capturés en nature et les saumons F1 laissent penser qu'une expérience de vie différente peut jouer un rôle significatif dans les comportements d'évitement des prédateurs. De plus, les différences observées entre les saumons F1 et F2 font croire que même une seule génération complète d'élevage en pisciculture peut suffire pour sélectionner des réactions mal adaptées à la prédation dans les conditions naturelles. Compte tenu de la controverse au sujet de l'efficacité des programmes d'élevage en pisciculture pour des fins de conservation, les résultats de notre étude indiquent que la diminution maximale du temps en pisciculture peut réduire les différences comportementales entre les poissons élevés en nature et ceux de pisciculture.

[Traduit par la Rédaction]

Introduction

In response to declining fish populations, hatchery programs have been implemented to supplement fish already present in the wild (Kostow 2009). However, there has been much criticism on the effectiveness of hatchery programs in increasing wild population stocks, and these programs have been the focus of much research (Waples 1999; Fraser 2008; McClure et al. 2008). Hatchery-reared fishes, ranging from fry to the adult life stage, have been shown to have reduced foraging efficiency (Bachman 1984; Reiriz et al. 1998), reduced swimming performance (Duthie 1987), reduced reproductive success (Fleming and Gross 1993; Berejikian et al. 2001), increased aggression (Mesa 1991; Deverill et al. 1999), and a reduced antipredator response when compared with their wild counterparts (Johnsson 1993; Álvarez and Ni-

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cieza 2003). Since predation is one of the major causes of mortality in hatchery-released fishes (Olla et al. 1998), these behavioural differences are thought to reduce survival and overall fitness of hatchery-reared fishes when they are released into the wild (Biro et al. 2004; Fraser 2008).

Given the importance of animal behaviour in conservation (Sutherland 1998; Caro 1999), it is of concern that many hatchery-reared salmonid species exhibit a reduced antipredator response when exposed to a predator (reviewed in Huntingford 2004). This reduced antipredator response may result from genetic divergence, because natural selection pressures are altered or relaxed during the process of captive rearing (Brown and Laland 2001), resulting in the inadvertent selection of traits that are beneficial in the hatchery but maladaptive in the wild (Olla et al. 1998; Seddon et al. 2007). In addition, owing to pronounced differences between the environmental conditions of hatchery and wild environments, the unnatural hatchery setting may not allow for individuals to acquire essential learned behaviours (experience), such as predator recognition and avoidance (Olla et al. 1998), and therefore individuals may be behaviourally ill-equipped to respond to predators in the wild (Brown and Day 2002). Thus, these documented differences in predator avoidance patterns may be due to "hatchery-selection" effects, differential experience, or some combination of the two.

Despite much research into the effects of the hatchery environment on salmonid antipredator behaviour, studies have typically compared multigenerational (>five generations) hatchery-reared salmonids to wild-caught conspecific salmonids or strains originating from differing (nonlocal) populations (Fraser 2008). Only three laboratory-based studies have compared the effects of hatchery rearing on predator avoidance between wild and first- and (or) second-generation hatchery-reared salmonid fry (*Salmo trutta*, Dellefors and Johnsson 1995; Álvarez and Nicieza 2003; *Oncorhynchus tshawytscha*, Fritts et al. 2007). However, only two studies (Dellefors and Johnsson 1995; Fritts et al. 2007) tested fish originating from the same source population. Given the strong effects of population-specific differences in behaviour patterns within salmonids, tests of potential hatchery-selection effects should ideally be conducted on wild-caught and hatchery strains originating from the same population (Fraser 2008).

The main objective of this study was to determine if there is a difference in antipredator behaviour between wild-caught and hatchery-reared juvenile (0+) Atlantic salmon (*Salmo salar*) under ecologically realistic (seminatural) conditions. We compared four behaviours associated with an antipredator response in local populations of wild-caught and hatchery-reared fry bred for up to two generations in a hatchery. Individual 0+ salmon of each strain were exposed to conspecific damage-released chemical alarm cues (or a stream water control). Conspecific alarm cues are known to elicit dramatic, short-term increases in predator avoidance behaviour in juvenile Atlantic salmon under natural conditions (Leduc et al. 2006; Kim et al. 2009). The secondary objective of this study was to predict the mechanism underlying any differences in antipredator behaviour. We predicted that if prior experience is the sole factor affecting antipredator behaviour, then first- and second-generation hatchery fish should show a similar reduced-intensity antipredator response compared with wild-caught salmon. Conversely, if hatchery selection is the only mechanism influencing antipredator behaviour, then we predicted that wild-caught and first-generation hatchery-reared fish should display a similar antipredator response, with the second-generation hatchery fish showing a reduced response (Álvarez and Nicieza 2003). However, if hatchery selection and prior experience both have an effect on antipredator behaviour, then we predicted that fry reared in the hatchery over two generations should show the most reduced antipredator response, followed by first-generation hatchery fry and then the wild-caught salmon. This study is unique in that comparing local populations of wild-caught and first- and second-generation hatchery-reared fry under seminatural conditions will allow us to differentiate between the effects of hatchery selection and prior experience on the observed antipredator response.

Materials and methods

Test site

This experiment was conducted in the Little Southwest Miramichi River located in Northumberland County, New Brunswick, Canada, over 2 consecutive years between 6 July and 9 August 2009 and between 4 and 23 July 2010. Two test sites were chosen approximately 100 m downstream from the mouth of Catamaran Brook. The two test sites, each containing one plastic testing bin, were 25 m apart and were chosen owing to their similar physical characteristics.

Before the start of the first trial, the pH and water temperature were recorded (using a portable Accumet pH meter and Hanna waterproof pH tester, in 2009 and 2010, respectively), as well as water velocity (measured at 50% of water column depth using a Flo-Mate velocity meter), depth (using a 1 m ruler), air temperature, and cloud cover. The pH, water velocity, and depth were measured once a day, whereas air temperature, water temperature, and cloud cover were measured every fourth trial. The water velocity at each test site was measured inside each enclosure. Cloud cover was measured by estimating the amount of cover directly over the test site using an eight-square grid, with zero being no cover and eight being full cloud cover, which was later converted into percent coverage.

Test fish

All wild juvenile (young-of-year, YOY) Atlantic salmon were captured from Rocky Brook with dip nets via snorkelling. Rocky Brook is a tributary of the Main Southwest Miramichi River and is located approximately 50 km from the testing location. First- and second-generation hatchery-reared juvenile (YOY) Atlantic salmon originating from Rocky Brook were obtained from the Miramichi Salmon Association (MSA) hatchery located in South Esk, New Brunswick, approximately 45 km from the testing location. First-generation (F1) hatchery-reared salmon were defined as the offspring of wild-caught Rocky Brook parents used as broodstock, while second-generation (F2) salmon were the offspring whose parents (F1) spent their entire lives in captivity. Each fall wild adults are captured entering their native spawning stream and are used as broodstock by the MSA hatchery (M. Hambrook, Miramichi Salmon Association, South Esk, New Brunswick, personal communication, 2010). The adult broodstock collection from Rocky Brook included five females and five males.

Wild-caught salmon ranged from 32.0 to 44.0 mm in standard length, while F1 and F2 salmon ranged from 34.0 to 46.0 mm and 35.0 to 50.0 mm, respectively. F1 and F2 hatchery salmon emerged as fry in early June.

Wild and hatchery-reared fish were transported to the testing location in 100 L metal containers containing either brook or well water, respectively. Upon arrival at the test location, the fish were placed in transparent 99 L plastic holding bins (0.8 m × 0.46 m × 0.34 m) in Little Southwest Miramichi River to allow them to acclimatize to the more natural stream conditions. All fish spent a minimum of 24 h in the holding bins and up to a maximum of 3 days before testing. The front and back of the holding bins were cut out and replaced with 3.175 mm wire mesh to allow for more natural flow conditions. Substrate, consisting of small to medium-sized rocks, was added to the bottom of the bins to mimic the actual stream bed. To eliminate the chance of escape and predation, the holding bins were secured with a lid. In addition, the holding bins were tethered to a tree using rope to reduce the risk of their being displaced by the current. The two testing bins had identical dimensions and conditions as the holding bins except that they did not have lids. Transportation and acclimation time was similar for all three strains, so it is unlikely that differential stress responses would be a confounding factor.

Stimulus preparation

Skin from F1 hatchery-reared juvenile Atlantic salmon was collected as the damaged released chemical alarm cue stimulus on 7 July 2009 and 6 July 2010. In 2009, parr (1+) were used, while in 2010, we used fry (0+), as the MSA hatchery did not have any parr (1+) available for sacrifice that year. Previous work on the Atlantic salmon alarm cue has demonstrated that there is no evidence of age-specific (0+ versus 1+) responses to alarm cue (Kim et al. 2009). Immediately following their removal from the hatchery tanks, the fish were killed humanely with a single blow to the head (in accordance with Concordia Animal Care Committee Protocol AREC-2008-BROW). In 2009, skin fillets were removed from both sides of the fish and directly placed in an ice-chilled beaker filled with untreated neutral well water. In 2010, owing to the small size of the donor fish, the entire body (skin and skeletal tissue), excluding the head, tail, and internal visceral organs, was used as the source of alarm cue and placed in an ice-chilled beaker filled with untreated neutral well water. Previous work has shown that whole-body homogenates are a reliable indicator of predation risk, can elicit an antipredator response, and therefore function as an alarm cue (Brown and Smith 1997). The skin fillets and body were homogenized, filtered through polyester filter floss, and diluted with untreated well water, resulting in alarm cue solutions of 0.1038 $cm^2 \cdot mL^{-1}$ for both years. While the exact alarm cue concentration that the fish is exposed to is unknown, it has been shown that similar substances in similar conditions (flow rate, water depth) can persist in the water column for sufficient time to elicit a behavioural response (Kim et al. 2011). Alarm cue solutions of 0.06 $cm^2 \cdot mL^{-1}$ and above have been shown to elicit a predictable antipredator response in salmonids under fully natural conditions (Leduc et al. 2006; Kim et al. 2009). The solution was frozen in 60 mL aliquots at –20 °C until needed. For this study, 2320 mL of alarm cue (240.82 cm^2 of skin) was used, while the rest was used for other ongoing studies. The frozen solutions were thawed 1 h prior to use. This preparation procedure has been shown to elicit an antipredator response in a wide range of fish taxa, including salmonids (Brown and Smith 1998; Kim et al. 2009).

Experimental protocol

Field observations were conducted between 22 July and 9 August 2009 and between 12 and 22 July 2010. When transported from a holding bin into a testing bin, the fish were allowed to acclimatize for approximately 25 min prior to testing. Trials were conducted by a single observer (Christopher Jackson) snorkelling adjacent to the testing bin. Testing consisted of 20 min trials divided into a 10 min prestimulus and a 10 min poststimulus observation period. After the prestimulus observation period, the observer injected 20 mL of the stimulus (either alarm cue or stream water for the experimental or control treatments, respectively) using a 60 mL syringe. The stimulus was injected at mid-water depth and approximately 10 cm upstream of the wire mesh front opening to not disturb the test fish. After each individual trial, standard length was recorded and the fish was released approximately 50 m downstream from the test site furthest downstream. The testing bins were removed at the end of the day and placed on shore.

We tested a total of 11 wild-caught salmon per chemical stimulus ($n = 22$), 15 F1 salmon per stimulus ($n = 30$), and 30 F2 salmon per stimulus ($n = 60$) for an overall sample size of $n = 112$. F1 salmon were not available from the MSA hatchery during 2010, and we were unable to capture wild YOY salmon in Rocky Brook during the 2009 field season. F2 salmon were tested in both 2009 and 2010.

Behavioural measures

To accurately assess the intensity of an antipredator response from direct exposure to a stimulus, four behaviour measures were recorded during each trial. Time spent moving (in seconds), time spent on substrate (in seconds), and the number of foraging attempts were recorded every minute for the duration of each trial. Latency to resume foraging (in seconds) was recorded following injection of the alarm cue stimulus only. These behavioural measures were chosen because they allow us to quantify the intensity of an antipredator response in juvenile Atlantic salmon (Leduc et al. 2007; Kim et al. 2009) and may confer increased survival benefits to individuals displaying these antipredator response behaviours during live predator encounters (Mathis and Smith 1993; Mirza and Chivers 2001).

Time spent moving was measured when an individual changed its location by at least half a body length over a period of 5 s. Time spent on substrate was measured when an individual was resting motionless on the substrate bottom. A foraging attempt was defined as the movement of at least half a body length with a biting attempt toward a food item in the water column or on the substrate floor. Latency to resume foraging was defined as the time elapsed before an individual captured its first food item following exposure to an alarm cue stimulus.

Statistical analysis

For time moving, time on the substrate and the frequency

Table 1. Mean value (± standard error, SE) and range of the physical variables in 2009 and 2010.

	2009		2010	
Variable	Mean ± SE	Range	Mean ± SE	Range
Water velocity ($cm \cdot s^{-1}$)	22.9±1.00	11.0–33.0	17.1±1.91	7.0–27.0
Depth (cm)	18.9±0.50	14.0–25.0	19.2±0.63	15.0–24.0
pH	7.15±0.03	6.59–7.37	7.80±0.01	7.76–7.88
Cloud cover (%)	51.13±4.75	0–100	48.38±6.00	0–100
Water temperature (°C)	16.4±0.13	15.0–19.0	21.5±0.36	18.0–26.5

Note: For each year, both test sites were pooled together.

of foraging attempts, we calculated the change in each behaviour from the prestimulus to the poststimulus observation period and used these difference scores as dependant variables in all subsequent analyses. Since all behavioural measures exhibited unequal variances, analyses were conducted on ranked difference scores. This nonparametric approach has been extensively used in the past (Brown et al. 2009), is robust, and represents a conservative estimate of parametric tests (Quinn and Keough 2002). Given that time moving, time on the substrate, and frequency of foraging attempts are likely highly correlated, we used a multivariate approach to account for dependency among our behavioural measures. Initially, we employed a multivariate generalized linear model (GLM, multivariate analysis of variance (MANOVA)), with the rank scores for each behavioural measure as dependant variables and strain (wild, F1, or F2) and chemical stimulus (alarm cue vs. distilled water) as independent variables. Given the significant strain × chemical stimulus interaction (see below), we conducted subsequent MANOVAs for salmon exposed to stream water and alarm cue separately. Simple planned contrasts were used to test for differences between strains in the event of significant main effects (Quinn and Keough 2002).

For latency to resume foraging, only the values for the alarm cue treatment were recorded in the field owing to the fact that we were interested in comparing their response to the alarm cue and not to stream water. If an individual fish did not resume foraging during the poststimulus observational period, that fish received a score of 600 s. The data were non-normally distributed, and therefore we used the rank scores as the dependant variable (Quinn and Keough 2002). We conducted a simple planned contrast using strain as the independent variable.

To test for potential differences in baseline values for time moving, time on the substrate, and frequency of foraging, we compared the prestimulus scores for each strain using a multivariate GLM (MANOVA). Planned contrasts were used to test for differences between strains in the event of a significant main effect.

Results

Habitat variables

Owing to a change in weather conditions between summer 2009 and 2010, some of the physical variables measured were not identical between years (Table 1). However, most variables had similar ranges and fell within the normal range for preferred Atlantic salmon habitat within the Little Southwest Miramichi River basin (Cunjak et al. 1993; Breau et al. 2007).

Behavioural variables

We found that year (2009, 2010) had no significant effect on salmon antipredator behaviour (MANOVA: $F_{[3,54]} = 1.67$, $P = 0.183$), while there was a significant effect of stimulus ($F_{[3,54]} = 8.52$, $P < 0.001$) and no significant interaction ($F_{[3,54]} = 1.02$, $P = 393$). We were only able to compare the antipredator response of F2 salmon because it was the only strain that was tested in both years.

Baseline activity levels (time spent moving, time on the substrate, and frequency of foraging attempts) were significantly different among strains (MANOVA: $F_{[3,108]} = 16.97$, $P < 0.001$; Fig. 1). We found significant planned contrasts for two of three behavioural measures (time on the substrate and frequency of foraging attempts; $P < 0.001$ for both). Wild salmon spent less time on the substrate and foraged at higher rates during the prestimulus observation periods compared with F1 and F2 salmon (Figs. 1*b* and 1*c*). We found no difference in the prestimulus time spent moving among strains ($P = 0.17$; Fig. 1*a*).

Our overall MANOVA revealed significant main effects of strain ($F_{[3,105]} = 3.52$, $P = 0.017$) and chemical stimulus ($F_{[3,104]} = 25.22$, $P < 0.001$) on salmon antipredator behaviour. Moreover, we found a significant interaction between the two main effects ($F_{[3,105]} = 2.74$, $P = 0.047$). To further examine the significant interaction, we tested salmon exposed to stream water and alarm cue separately. We found no effect of strain on the response of salmon exposed to the stream water control ($F_{[3,52]} = 0.65$, $P = 0.588$). However, we did find a significant effect of strain for salmon exposed to alarm cue ($F_{[3,52]} = 6.37$, $P = 0.001$). Planned contrasts revealed that when exposed to alarm cue, wild salmon exhibited the strongest reduction in time spent moving and frequency of foraging attempts, and F2 exhibited the weakest (Figs. 2*a* and 2*c*). Interestingly, the F1 salmon exhibited a mixed pattern in which the reduction in time moving was not different from that of wild salmon (Fig. 2*a*), while the reduction in foraging attempts was (Fig. 2*c*), suggesting that F1 salmon were intermediate to wild and F2 salmon in their response to alarm cues. We found no difference among strains for salmon exposed to alarm cue for the increase in time spent on the substrate (i.e., all strains showed similar response patterns, Fig. 2*b*). Finally, we found a significant effect of strain on the latency to resume foraging ($F_{[2,52]} = 8.22$, $P = 0.001$). The latency to resume foraging was significantly longer for wild-caught versus F1 and F2 salmon (Fig. 3).

Discussion

Overall, the present findings suggest that while wild-caught and hatchery-reared juvenile Atlantic salmon exhib-

Fig. 1. Rank of baseline (prestimulus observation periods) time spent moving (*a*), time on the substrate (*b*), and foraging attempts (*c*) for wild, F1, and F2 salmon. Values are means ± standard error. Different letters denote significant differences ($P < 0.05$) based on simple planned contrasts.

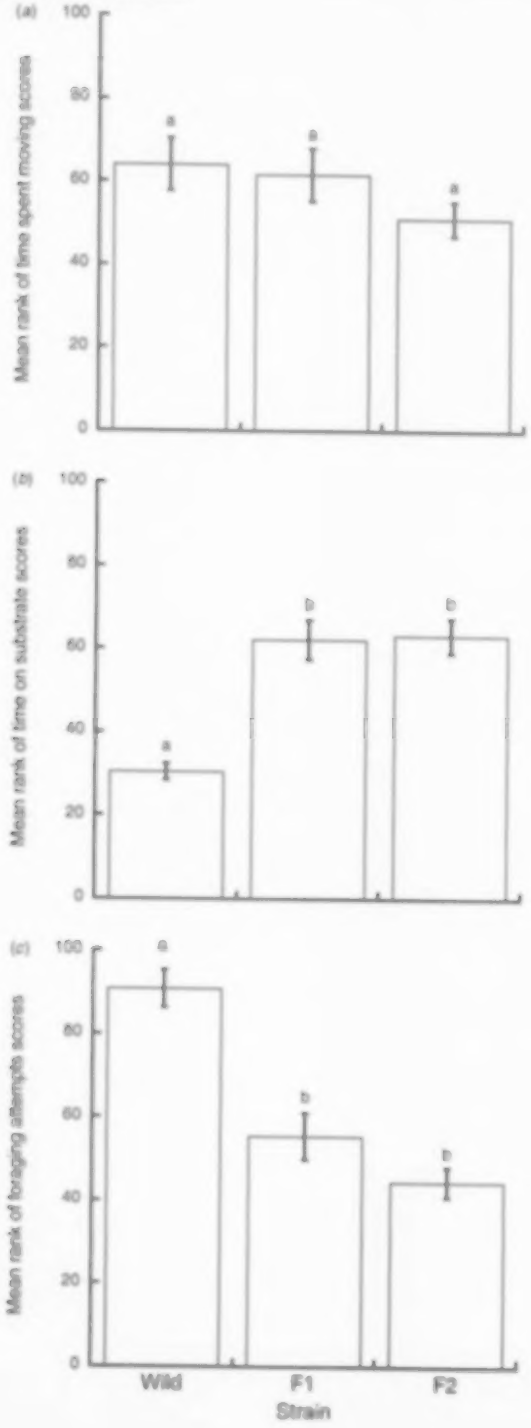


Fig. 2. Rank of difference scores (poststimulus – prestimulus values) for time spent moving (*a*), time spent on substrate (*b*), and foraging attempts (*c*) for wild, F1, and F2 salmon. Open bars denote salmon exposed to stream water; solid bars denote salmon exposed to conspecific alarm cues. Values are means ± standard error. Different letters above the alarm cue (solid) bars denote significant differences ($P < 0.05$) based on simple planned contrasts.

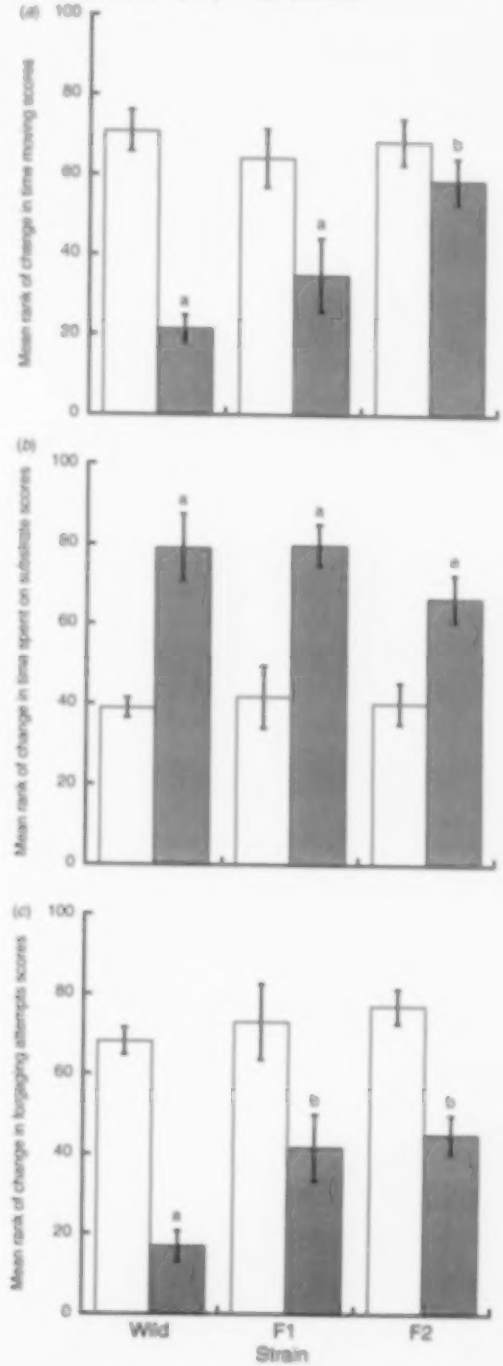
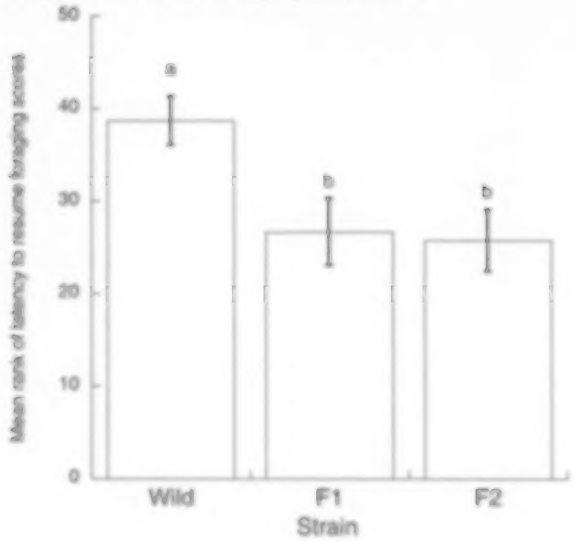


Fig. 3. Rank in latency to resume foraging scores for wild, F1, and F2 salmon following exposure to conspecific alarm cues. Values are means ± standard error. Different letters denote significant differences ($P < 0.05$) based on simple planned contrasts.



ited some response to conspecific alarm cues, there was a stronger response among wild-caught fry and a weaker response among hatchery-reared fry. Moreover, the response pattern of F1 salmon appears to be intermediate between that of wild-caught and F2 strains. These results are consistent with those of previous studies, which have shown that both hatchery-reared and predator naive salmonids display innate antipredator responses to predation risk (Brown and Smith 1998; Berejikian et al. 1999; Scheurer et al. 2007). Moreover, extensive research conducted by Leduc et al. (2006, 2007, 2009) has demonstrated that wild juvenile Atlantic salmon display a similar antipredator response when exposed to conspecific alarm cues to that shown here.

One interesting finding was that wild-caught salmon had the highest baseline foraging rates compared with F1 and F2 salmon. This result is consistent with that of other studies, which have demonstrated that hatchery-reared salmonids display a reduced foraging efficiency when exposed to live novel prey items compared with their wild counterparts (Ersbak and Haase 1983; Bachman 1984; Sundström and Johnsson 2001). This may be because hatchery-reared salmon have not been previously exposed to live prey items and therefore lack the appropriate experience to select proper food items (Brown et al. 2003). There is, however, conflicting evidence that suggests that prior experience of live prey has no impact on the postrelease foraging behaviour of hatchery-reared salmonids (Massee et al. 2007). A more comprehensive study comparing foraging rates between wild-caught and hatchery-reared salmon over an extended period of time under natural conditions would be needed to draw any definite conclusions.

More importantly, this study suggests that wild-caught and first- and second-generation hatchery-reared salmon display antipredator responses of dissimilar intensities. Wild-caught salmon displayed a greater reduction in foraging and resumed foraging later than their F1 counterparts when exposed to alarm cue. In addition, F1 salmon exhibited a greater reduction in time moving compared with F2 salmon following predator exposure. Overall, the response pattern of F1 salmon was intermediate between that of wild-caught and F2 salmon, suggesting that both differential experience and genetic selection may play an important role in the observed difference in predator avoidance patterns of hatchery-reared salmon.

A proposed mechanism widely cited in current literature that may account for these behavioural differences is the role of previous predator experience in shaping antipredator responses to predation risk. A hatchery setting lacks many of the natural aspects of a wild environment, most importantly predators, and as a result hatchery-reared individuals may not develop the acquired predator recognition and avoidance behaviours needed to survive in the wild (Brown and Day 2002). Consequently, the survival rate of hatchery-reared fishes into adulthood is extremely poor, and this reduces the usefulness of hatchery programs (Brown and Laland 2001). Berejikian (1995) found that during live predator encounters, wild (experienced) steelhead trout fry survived significantly better than their hatchery-reared (predator naïve) counterparts. Similarly, hatchery-reared (predator experienced) juvenile coho salmon (*Oncorhynchus kisutch*) consistently survived in greater numbers and for longer periods of time when exposed to a live lingcod (*Ophiodon elongates*) than did hatchery-reared (predator naïve) juvenile coho salmon (Olla and Davis 1989). In addition, Álvarez and Nicieza (2003) found that wild and first-generation hatchery-reared juvenile brown trout differ in their predator avoidance behaviour, with the F1 individuals using refuging less than their wild counterparts, which they argued could be explained by previous experience with predators. However, two of these studies, Berejikian (1995) and Olla and Davis (1989), used multigenerational hatchery-reared individuals, while Álvarez and Nicieza (2003) used fish from different donor stocks. It is crucial to highlight the importance of using test fish from the same source population to eliminate population differences, and after limited hatchery exposure (one to two generations), because this strategy is commonly used to limit behavioural differences between the hatchery-reared and wild individuals (Fritts et al. 2007).

Another proposed mechanism that may explain behavioural differences in hatchery-reared and wild salmonids is domestication selection. Much of the literature has suggested that increased culture time (rearing over multiple generations), artificial selection, and the relaxation of natural selection pressures have resulted in dramatic antipredator behaviour differences between cultured and wild individuals (Huntingford 2004). Many of the studies looking into the effects of domestication on antipredator behaviour have focused either on multigenerational (more than three to four generations) artificially cultured salmonids (Johnsson et al. 1996; Petersson and Jarvi 2006; Houde et al. 2010) or situations where there was strong intentional selection for faster-growing salmonids used in aquaculture (Johnsson and Abrahams 1991; Fleming and Einum 1997; Yamamoto and Reinhardt 2003). Only one previous laboratory study, testing survival directly (Fritts et al. 2007), has been conducted between first- and second-generation hatchery-reared individuals; these individualas were obtained from the same donor stock, thereby eliminating the effect of population differences. Fritts et al.

(2007) found that F1 hatchery-reared juvenile Chinook salmon (*O. tshawytscha*) had a 2.2% survival advantage compared with the F2 generation when exposed to a live predator, indicating that domestication can affect the vulnerability to predators after only one generation of hatchery rearing. The effect, however, was small and would likely have little impact on population stability. Any domestication selection observed in this study would most likely be due to relaxed predation selection pressure found in the hatchery and not artificial selection because the hatchery does not intentionally select for fast-growing individuals (M. Hambrook, Miramichi Salmon Association, South Esk, New Brunswick, personal communication, 2010). There may, however, be unintentional selection occurring (e.g., growth) that may be unaccounted for.

We must be cautious, however, in stating that the observed differences in antipredator behaviour between hatchery-reared and wild salmon are solely due to previous experience and (or) domestication selection. Owing to our experimental design, it is impossible for us to rule out other alternative mechanisms, including early life-history selection. Since our strains were not reared under the same common conditions, there was probably some size selection on the wild eggs and (or) juveniles, which would not occur in the hatchery. Studies have shown, for example, that large egg and juvenile size are selected for in wild environments owing to the increased chance of over-winter survival (Einum and Fleming 1999, 2000). Therefore, the observed differences between the wild and F1 strains may not be solely due to previous experience but may also be due to selection pressures in the wild. Also, the wild fish tested have already undergone selection (selective mortality) prior to the experiment, leading to individuals with maladaptive antipredator behaviour being selected out of the population, and resulting in wild fish with increased antipredator responses compared with the hatchery fish. In addition, since the F1 and F2 progeny had parents that were reared under different conditions, there may have also been selection on the wild adults, which may have produced differences in behaviour between the F1 and F2 strains.

A second point of caution arises from the unequal testing design of our study. Owing to logistic constraints, we were unable to test all strains in both field seasons. However, year effects are unlikely to account for our observed results for at least two reasons. Initially, while physical (habitat) variables did differ between years, salmon were always tested in what would be considered normal habitat for YOY. When we compared F2 fish tested in 2009 and 2010, no effect of year on any behavioural measure was found. Thus, while we cannot completely rule out the effect of year, it is unlikely the driving factor in our observed results.

From a conservation standpoint, it is important to mention what this specific hatchery program does or does not do to minimize behavioural differences between their hatchery-reared fish and wild counterparts. The first and probably most important is the use of local strains in all their hatchery fish populations. Releasing local strains back into the wild may help in reducing behavioural differences because these fish are locally adapted to their environment. Programs that use nonlocal strains to supplement wild fish populations should take into consideration local adaptations and the effect of these adaptations on behaviour and fitness. Second, typical juvenile densities in the hatchery range from 5 to 15 $kg \cdot m^{-3}$, which is much higher than observed in the wild but is typical of most hatchery programs. Third, all fish are released as juveniles, either as first feeding fry (0.3 g) or fall fingerlings (6 g), thus minimizing the time spent in the hatchery and reducing the opportunity for selection to occur.

One aspect that this program does not focus on is the artificial rearing conditions and the effect they may have on the behavioural deficiencies of hatchery-reared fish. All fish are fed artificial feed from overhead, and the tanks do not include any form of complexity. Structures such as natural substrate and rocks and branches, underwater feeders, and the addition of live prey items, overhead cover, and the use of alarm cues or predator odours can all be used to add complexity to a hatchery rearing environment. For example, increasing habitat complexity (in-water structures, underwater feeders, and live prey) has been shown to modify the behaviour of hatchery-reared salmonids, which may significantly improve their postrelease survival rates (Berejikian et al. 2000; Brown et al. 2003). In addition, the use of conspecific chemical alarm cues and predator odour may be used to condition hatchery (predator-naïve) fish to show an appropriate antipredator response when fish are exposed to predator odour, which may improve survival in the wild (Brown and Smith 1998; Berejikian et al. 1999).

We need a better understanding of how the hatchery environment and incorporated strategies affect the behaviour and postrelease survival of hatchery fishes in order for hatcheries to increase in efficiency and fulfill their objective. More studies are needed, ideally under natural conditions, directly comparing wild, F1, and F2 generations from the same population to help disentangle the effects of experience and hatchery selection on behaviour and survival of hatchery-reared fishes.

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References

Álvarez, D., and Nicieza, A.G. 2003. Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. J. Fish Biol. **63**(6): 1565–1577. doi:10.1111/j.1095-8649.2003.00267.x.

Bachman, R.A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Trans. Am. Fish. Soc. **113**(1): 1–32. doi:10.1577/1548-8659(1984)113<1:FBOFWA>2.0.CO;2.

Berejikian, B.A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Can. J. Fish. Aquat. Sci. **52**(11): 2476–2482. doi:10.1139/f95-838.

Berejikian, B.A., Smith, R.J.F., Tezak, E.P., Schroder, S.L., and Knudsen, C.M. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. Can. J. Fish. Aquat. Sci. **56**(5): 830–838. doi:10.1139/f99-010.

Berejikian, B.A., Tezak, E.P., Flagg, T.A., LaRae, A.L., Kummerow, E., and Mahnken, C.V.W. 2000. Social dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. Can. J. Fish. Aquat. Sci. **57**(3): 628–636. doi:10.1139/f99-288.

Berejikian, B.A., Tezak, E.P., and Schroder, S.L. 2001. Reproductive behavior and breeding success of captively reared Chinook salmon. N. Am. J. Fish. Manage. **21**(1): 255–260. doi:10.1577/1548-8675(2001)021<0255:RBABSO>2.0.CO;2.

Biro, P.A., Abrahams, M.V., Post, J.R., and Parkinson, E.A. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc. Biol. Sci. **271**(1554): 2233–2237. doi:10.1098/rspb.2004.2861. PMID:15539348.

Breau, C., Cunjak, R.A., and Bremset, G. 2007. Age-specific aggregation of wild juvenile Atlantic salmon *Salmo salar* at cool water sources during high temperature events. J. Fish Biol. **71**(4): 1179–1191. doi:10.1111/j.1095-8649.2007.01591.x.

Brown, C., and Day, R.L. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish Fish. **3**(2): 79–94.

Brown, C., and Laland, K. 2001. Social learning and life skills training for hatchery reared fish. J. Fish Biol. **59**(3): 471–493. doi:10.1111/j.1095-8649.2001.tb02354.x.

Brown, G.E., and Smith, R.J.F. 1997. Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool. **75**(11): 1916–1922. doi:10.1139/z97-821.

Brown, G.E., and Smith, R.J.F. 1998. Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery reared fish to recognize chemical cues of a predator. Can. J. Fish. Aquat. Sci. **55**(3): 611–617. doi:10.1139/f97-261.

Brown, C., Davidson, T., and Laland, K. 2003. Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. J. Fish Biol. **63**(s1 Suppl. A): 187–196. doi:10.1111/j.1095-8649.2003.00208.x.

Brown, G.E., Harvey, M.C., Leduc, A.O.H.C., Ferrari, M.C.O., and Chivers, D.P. 2009. Social context, competitive interactions and the dynamic nature of antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). J. Fish Biol. **75**(3): 552–562. doi:10.1111/j.1095-8649.2009.02305.x. PMID:20738556.

Caro, T. 1999. The behaviour-conservation interface. Trends Ecol. Evol. **14**(9): 366–369. doi:10.1016/S0169-5347(99)01663-8. PMID:10441313.

Cunjak, R.A., Caissie, D., El-Jabi, N., Hardie, P., Conlon, J.H., Pollock, T.L., Giberson, D.J., and Komadina-Douthwright, S. 1993. The Catamaran Brook (New Brunswick) Habitat Research Project: biological, physical and chemical conditions (1990–1992). Can. Tech. Rep. Fish. Aquat. Sci. No. 1914.

Deflefors, C., and Johnsson, J.I. 1995. Foraging under risk of predation in wild and hatchery-reared juvenile sea trout (*Salmo trutta* L.). Nord. J. Freshw. Res. **0**(70): 31–37.

Deverill, J.I., Adams, C.E., and Bean, C.W. 1999. Prior residence, aggression and territory acquisition in hatchery-reared and wild brown trout. J. Fish Biol. **55**(4): 868–875. doi:10.1111/j.1095-8649.1999.tb00723.x.

Duthie, G.G. 1987. Observations of poor swimming performance among hatchery-reared rainbow trout, *Salmo gairdneri*. Environ. Biol. Fishes, **18**(4): 309–311. doi:10.1007/BF00004884.

Einum, S., and Fleming, I.A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proc. Biol. Sci. **266**(1433): 2095–2100. doi:10.1098/rspb.1999.0893.

Einum, S., and Fleming, I.A. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution, **54**(2): 628–639. PMID:10937238.

Ersbak, K., and Haase, B.L. 1983. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. N. Am. J. Fish. Manage. **3**(2): 142–151. doi:10.1577/1548-8659(1983)3<142:NDASAA>2.0.CO;2.

Fleming, I.A., and Einum, S. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES J. Mar. Sci. **54**(6): 1051–1063. doi:10.1016/S1054-3139(97)80009-4.

Fleming, I.A., and Gross, M.R. 1993. Breeding success of wild and hatchery coho salmon (*Oncorhynchus kisutch*) in competition. Ecol. Appl. **3**(2): 230–245. doi:10.2307/1941826.

Fraser, D.J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evol. Appl. **1**(4): 535–586.

Fritts, A.L., Scott, J.L., and Pearsons, T.N. 2007. The effects of domestication on the relative vulnerability of hatchery and wild origin spring Chinook salmon (*Oncorhynchus tshawytscha*) to predation. Can. J. Fish. Aquat. Sci. **64**(5): 813–818. doi:10.1139/f07-057.

Houde, A.L.S., Fraser, D.J., and Hutchings, J.A. 2010. Reduced antipredator responses in multi-generational hybrids of farmed and wild Atlantic salmon (*Salmo salar* L.). Conserv. Genet. **11**(3): 785–794. doi:10.1007/s10592-009-9892-2.

Huntingford, F.A. 2004. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. J. Fish Biol. **65**(s1 Suppl. A): 122–142. doi:10.1111/j.0022-1112.2004.00562.x.

Johnsson, J.I. 1993. Big and brave: size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. **45**(6): 1219–1225. doi:10.1006/anbe.1993.1143.

Johnsson, J.I., and Abrahams, M.V. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Can. J. Fish. Aquat. Sci. **48**(2): 243–247. doi:10.1139/f91-033.

Johnsson, J.I., Petersson, E., Jonsson, E., Bjornsson, B.H., and Jarvi, T. 1996. Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, *Salmo trutta*. Can. J. Fish. Aquat. Sci. **53**(7): 1546–1554. doi:10.1139/f96-090.

Kim, J.-W., Brown, G.E., Dolinsek, I.J., Brodeur, N.N., Leduc, A.O.H.C., and Grant, J.W.A. 2009. Combined effects of chemical and visual information in eliciting antipredator behaviour in juvenile Atlantic salmon *Salmo salar*. J. Fish Biol. **74**(6): 1280–1290. doi:10.1111/j.1095-8649.2009.02199.x. PMID:20735631.

Kim, J.-W., Grant, J.W.A., Brown, G.E., and Fleming, I. 2011. Do juvenile Atlantic salmon (*Salmo salar*) use chemosensory cues to detect and avoid risky habitats in the wild. Can. J. Fish. Aquat. Sci. **68**(4): 655–662. doi:10.1139/f2011-011.

Kostow, K. 2009. Factors that contribute to the ecological risks of salmon and steelhead hatchery programs and some mitigating strategies. Rev. Fish Biol. Fish. **19**(1): 9–31. doi:10.1007/s11160-008-9087-9.

Leduc, A.O.H.C., Roh, E., Harvey, M.C., and Brown, G.E. 2006. Impaired detection of chemical alarm cues by juvenile wild Atlantic salmon (*Salmo salar*) in a weakly acidic environment. Can. J. Fish. Aquat. Sci. **63**(10): 2356–2363. doi:10.1139/f06-128.

Leduc, A.O.H.C., Roh, E., Breau, C., and Brown, G.E. 2007. Effects of ambient acidity on chemosensory learning: example of an environmental constraint on acquired predator recognition in wild

juvenile Atlantic salmon (*Salmo salar*). Ecol. Freshwat. Fish. **16**(3): 385–394. doi:10.1111/j.1600-0633.2007.00233.x.

Leduc, A.O.H.C., Roh, E., and Brown, G.E. 2009. Effects of acid rainfall on juvenile Atlantic salmon (*Salmo salar*) antipredator behaviour: loss of chemical alarm cue function and potential survival consequences during predation. Mar. Freshw. Res. **60**(12): 1223–1230. doi:10.1071/MF08323.

Massee, K.C., Kim, J., Berejikian, B.A., and Hardy, R.W. 2007. Prey selection and efficiency of naïve and experienced juvenile sockeye salmon. J. Fish Biol. **70**(4): 1213–1223. doi:10.1111/j.1095-8649.2007.01389.x.

Mathis, A., and Smith, R.J.F. 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). Behav. Ecol. **4**(3): 260–265. doi:10.1093/beheco/4.3.260.

McClure, M.M., Utter, F.M., Baldwin, C., Carmichael, R.W., Hassemer, P.F., Howell, P.J., Spruell, P., Cooney, T.D., Schaller, H.A., and Petrosky, C.E. 2008. Evolutionary effects of alternative artificial propagation programs: implications for viability of endangered anadromous salmonids. Evol. Appl. **1**(2): 356–375. doi:10.1111/j.1752-4571.2008.00034.x.

Mesa, M.G. 1991. Variation in feeding, aggression, and position choice and hatchery and wild Cutthroat trout in an artificial stream. Trans. Am. Fish. Soc. **120**(6): 723–727. doi:10.1577/1548-8659(1991)120<0723:VIFAAP>2.3.CO;2.

Mirza, R.S., and Chivers, D.P. 2001. Chemical alarm signals enhance survival of brook charr (*Salvelinus fontinalis*) during encounters with chain pickerel (*Esox niger*). Ethology, **107**(11): 989–1005. doi:10.1046/j.1439-0310.2001.00729.x.

Olla, B.L., and Davis, M.W. 1989. The role of learning and stress in predator avoidance of hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles. Aquaculture, **76**(3–4): 209–214. doi:10.1016/0044-8486(89)90075-6.

Olla, B.L., Davis, M.W., and Ryer, C.H. 1998. Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. Bull. Mar. Sci. **62**(2): 531–550.

Petersson, E., and Jarvi, T. 2006. Anti-predator response in wild and sea-ranched brown trout and their crosses. Aquaculture, **253**(1–4): 218–228. doi:10.1016/j.aquaculture.2005.08.012.

Quinn, G.P., and Keough, M.J. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Reiriz, L., Nicieza, A.G., and Braña, F. 1998. Prey selection by experienced and naïve juvenile Atlantic salmon. J. Fish Biol. **53**(1): 100–114. doi:10.1111/j.1095-8649.1998.tb00113.x.

Scheurer, J.A., Berejikian, B.A., Thrower, F.P., Ammann, E.R., and Flagg, T.A. 2007. Innate predator recognition and fright response in related populations of *Oncorhynchus mykiss* under different predation pressure. J. Fish Biol. **70**(4): 1057–1069. doi:10.1111/j.1095-8649.2007.01367.x.

Seddon, P.J., Armstrong, D.P., and Maloney, R.E. 2007. Developing the science of reintroduction biology. Conserv. Biol. **21**(2): 303–312. doi:10.1111/j.1523-1739.2006.00627.x. PMID:17391180.

Sundström, L.F., and Johnsson, J.I. 2001. Experience and social environment influence the ability of young brown trout to forage on live novel prey. Anim. Behav. **61**(1): 249–255. doi:10.1006/anbe.2000.1593. PMID:11170714.

Sutherland, W.J. 1998. The importance of behavioural studies in conservation biology. Anim. Behav. **56**(4): 801–809. doi:10.1006/anbe.1998.0896. PMID:9790690.

Waples, R.S. 1999. Dispelling some myths about hatcheries. Fisheries (Bethesda, Md.), **24**(2): 12–21. doi:10.1577/1548-8446(1999)024<0012:DSMAH>2.0.CO;2.

Yamamoto, T., and Reinhardt, U.G. 2003. Dominance and predator avoidance in domesticated and wild masu salmon (*Oncorhynchus masou*). Fish. Sci. **69**(1): 88–94. doi:10.1046/j.1444-2906.2003.00591.x.

Large-scale and long-term decrease in fish growth following the construction of hydroelectric reservoirs

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Abstract: Hydroelectric reservoirs retain large volumes of water and have a global impact on sea level, elemental cycles, and biodiversity. Using data from a total of 90 historical and recent surveys in nine regulated and eight unregulated alpine and subalpine lakes, we show an additional large effect of reservoirs, i.e., that impoundment causes drastically decreased fish growth and thereby great negative consequences for inland fisheries in Scandinavia. Following a long period (40–65 years) after impoundment, the length and mass of Arctic charr (*Salvelinus alpinus*) of the single age class 4+ years was, on average, 35% and 72% lower, respectively, in impounded versus natural lakes in northern Scandinavia. The effect was stronger at higher altitudes and can be mitigated by addition of inorganic nutrients. We suggest that the decreased fish growth is a consequence of lowered ecosystem productivity, oligotrophication, caused by impoundment, resulting in erosion and loss of the littoral ecosystem as well as delayed flooding and leakage of nutrients from the riparian zone until after the growing season.

Résumé : Les réservoirs d'origine anthropique retiennent d'importants volumes d'eau et ont un impact global sur le niveau de la mer, les cycles des éléments et la biodiversité. À l'aide de données provenant d'un ensemble de 90 inventaires du passé et inventaires actuels dans neuf lacs alpins et subalpins munis de barrage et huit lacs sans barrage, nous démontrons un effet supplémentaire important des réservoirs, à savoir que les transformations en réservoir diminuent considérablement la croissance des poissons et qu'elles ont ainsi de sérieuses conséquences négatives sur les pêches intérieures de la Scandinavie. Après une longue période (40–65 ans) après la transformation en réservoir, les longueurs et les masses des ombles chevaliers (*Salvelinus alpinus*) de la seule classe d'âge 4+ sont respectivement 35 % et 72 % inférieures dans les lacs-réservoirs par rapport aux lacs naturels dans le nord de la Scandinavie. L'effet est plus marqué aux altitudes plus élevées et peut être mitigé par l'addition de nutriments inorganiques. Nous croyons que la croissance réduite des poissons est une conséquence de la production plus basse de l'écosystème, donc de l'oligotrophisation, causée par la transformation en réservoir qui produit une érosion et une perte de l'écosystème littoral, ainsi qu'une inondation retardée et une perte des nutriments dans la zone riveraine jusqu'après la saison de croissance.

[Traduit par la Rédaction]

Introduction

The majority of the large rivers of the world are subject to impoundment (Nilsson et al. 2005). Hydroelectric reservoirs retain large volumes of water and have a global impact on sea level (Chao et al. 2008), elemental cycles (Humborg et al. 1997; St. Louis et al. 2000), and biodiversity (Poff et al. 2007). In Scandinavia, most rivers are regulated for hydroelectric power production. In the catchments of the eight major regulated rivers in northern Sweden (41% of the total area of Sweden), there are 90 reservoirs larger than 1 km^2 that are classified as being substantially or heavily affected by the change in water amplitude according to current environmental quality criteria. These reservoirs cover 3200 km^2, corresponding to 22% of the regional lake area and 8% of the total Swedish lake area (data from Swedish Meteorological and Hydrological Institute). Likewise, in Norway, 6000 km^2 are affected, representing 40% of the Norwegian freshwater surface area (Aass 1984). Water level fluctuations in the Swedish reservoirs are on average 7 m, but can be as large as 35 m. Drawdown occurs mainly during winter, which results in a heavy erosion of the littoral zone. It has been suggested that regulated ecosystems will gradually become oligotrophic (i.e., biological production declines below pre-impoundment levels) owing to habitat destruction and altered hydrology (Stockner et al. 2000; Rydin et al. 2008). Accord-

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ingly, it has been reported from Norwegian (Aass 1984; Borgstrøm and Aass 2000) as well as Swedish (Nilsson 1973) reservoirs that fish enter a process of steady decrease only a few years after impoundment. However, evidence in support of long-term oligotrophication effects on biota is lacking. Here, we demonstrate that decreased fish growth is a long-term effect without recovery within time scales approaching the expected life span of hydroelectric reservoirs.

Material and methods

To assess the long-term effect of impoundment on fish growth, we compiled a data set on the length and mass of Arctic charr (*Salvelinus alpinus*) of the single age class 4+ years in regulated and unregulated alpine and subalpine Swedish lakes. The data cover a period of up to 65 years after impoundment. Data on age, length (total length up to a line drawn between the tips of the terminal fin brought together in a parallel manner), and wet mass of individual Arctic charr were obtained from a total of 90 test fishings in eight unregulated and nine regulated alpine and subalpine lakes in the catchments of the rivers Indalsälven, Ångermanälven, and Torne älv. The fish communities in these lakes are mainly dominated by Arctic charr and brown trout (*Salmo trutta*) or charr alone. (The location, area, regulation amplitude, and occurrence of other fish species and *Mysis relicta* are presented in Table S1.[1]) The fishing pressure in the lakes chosen in this study is considered low or very low by normal Swedish standards, especially nowadays, and exploitation in the catchments is almost negligible except for the dam buildings. Hence, impoundment is the major anthropogenic disturbance in these ecosystems. To the best of our knowledge, the data set includes all available data from large (>2 km^2) regulated and unregulated Swedish alpine and subalpine lakes in the aforementioned rivers. Test fishings in those lakes cover the period 1936–2007 and were performed by the Freshwater Institute of the Swedish Board of Fisheries or by ourselves (lakes Stora Mjölkvattnet and Burvattnet) (Rydin et al. 2008).

Fish were caught in August to October, first with cotton nets and later on with nylon nets, both with sections of different mesh sizes in preset combinations. Most importantly, the test fishings were carried out before fish spawning. Otherwise, the exact date for autumn test fishing was considered less important. The number of net nights varied with lake size and was usually between 40 and 64. However, for many of the early fisheries, data on number of nets are not available. Early cotton nets had seven different mesh sizes between 16.5 and 50 mm (i.e., 16.5, 22, 25, 30, 33, 38, and 50 mm) knot to knot, where the 30 and 33 mm nets occurred twice. In the early 1960s, cotton nets were replaced by transparent monofilament nylon nets, with the addition of two extra sections of mesh sizes 10 and 12.5 mm knot to knot. In 1968, the first version of a new bottom-set, multipanel net of survey type was introduced, which then gradually developed into survey nets of the Nordic type (Hammar and Filipsson 1985). Each of these nets is thus a combination of gill-net panels, each 3 m long × 1.5 m deep, with a total length of originally 37 m. The currently used Nordic type is 43 m long and contains additional panels of larger mesh size (60 and 75 mm knot to knot, respectively) (European Committee for Standardization 2005). To allow for safe comparisons, test fishings in revisited lakes were performed in the same way and the nets with various mesh sizes arranged the same way on the same sites once selected.

Age determinations were based on the reading of scales mounted between glass slides and read in a microfiche reader (Bell & Howell model VIII) (1936–1962) (Runnström 1955) or otolith readings (1963–2007) (Filipsson 1967). A direct comparison of the otolith and scale reading methods shows that correct age determination on scales can be done with a very high degree of certainty of the age classes 3+ to 5+, i.e., 97% on average (Appendix S1; Table S3)[1]. Here we show results for charr at age 4+ years. Based on observations from Lake Stora Mjölkvattnet of the response across multiple age classes to nutrient addition (G. Milbrink et al., unpublished data), we expected that the potential effects of impoundment on fish growth should be more clearly manifested at ages older than 3 years. Furthermore, since age determinations on scales from charr older than 5 years are less reliable, and 4+ years is the most commonly occurring age class in the captures, 4+ charr were chosen for our analysis, as was previously done by Runnström (1955, 1964). The relatively large sample sizes of 4+fish compared with that of fish from other age classes thus allowed higher precision in the estimation of means and variances. Prior to statistical analysis, the data set was divided into four categories: unregulated lakes plus regulated lakes from the preimpoundment period, and 1–7, 10–38, and 40–65 years after regulation, respectively. The categories after impoundment were chosen to reflect the positive inundation effects on fish growth (based on previous literature knowledge about expected duration of this phase), the intermediate-term decrease of fish growth, and long-term effects, respectively. For each lake and time period, the mean mass and length were calculated (Table S2).[1] The data were analysed with one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc tests at $\alpha = 0.05$ (Zar 1984). The ANOVA was run both with and without weighting proportional to sample size (number of observations), but results were only marginally different. Hence, we present only results from the unweighted analyses. Likewise, the results of the ANOVA were robust to changes of the limits for the time categories above. Within categories of unregulated and regulated lakes ≥10 years after regulation, the relationships between length and mass and altitude, lake area, and regulation amplitude were analysed by linear regression analysis (Zar 1984). Differences in catch per unit effort were tested using the Wilcoxon rank–sum test (Conover 1980).

Results and discussion

The size of charr decrease substantially over time in regulated lakes (Fig. 1; Table 1). This decrease started less than 10 years after impoundment and continued throughout the whole period without any sign of recovery (Figs. 1*b*, 1*d*). The mean mass was 49% and 72% lower in lakes 10–38 and 40–65 years after impoundment, respectively, compared with

[1]Supplementary data are available with the article through the journal Web site (http://www.nrcresearchpress.com/doi/suppl/10.1139/f2011-131).

Fig. 1. Length and wet mass of 4+ Arctic charr in unregulated and regulated Swedish alpine and subalpine lakes. Mean length of charr in 8 unregulated lakes (*a*), mean length of charr in 10 regulated lakes (*b*), mean mass of charr in unregulated lakes (*c*), and mean mass of charr in regulated lakes (*d*). Error bars show 95% confidence intervals of the mean, calculated from individual lengths and masses. Lengths and masses of charr in regulated lakes are shown over time, with the time axis normalized to show years after impoundment (year 0, vertical broken line). Horizontal solid lines show grand mean length and mass of charr in unregulated lakes, including preimpoundment data from regulated lakes, and the horizontal broken lines show 95% confidence intervals of the mean. Gray solid lines show smoothing spline fits (λ = 1000) based on all data, except those for Lake Stora Mjölkvattnet, after nutrient addition.

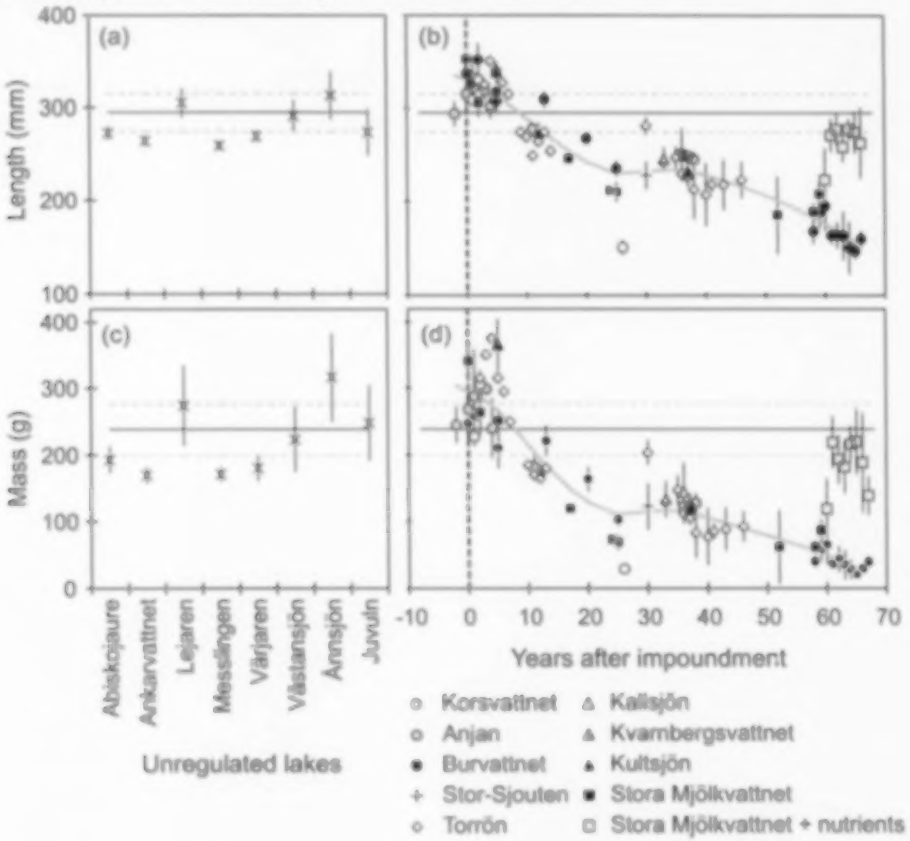


Table 1. Length and wet mass (mean ± 95% confidence interval) of 4+ Arctic charr in unregulated and regulated lakes.

Lake category	Length (mm)*	Mass (g)**	*n*
Unregulated or before regulation	295±20a	239±39a	11
1–7 years after regulation	324±10a	290±45a	6
10–38 years after regulation	236±27b	123±30b	9
40–65 years after regulation	193±62b	66±56b	3

Note: Categories not followed by same letter are significantly different (Tukey post hoc test at $\alpha = 0.05$). *n*, number of lakes.

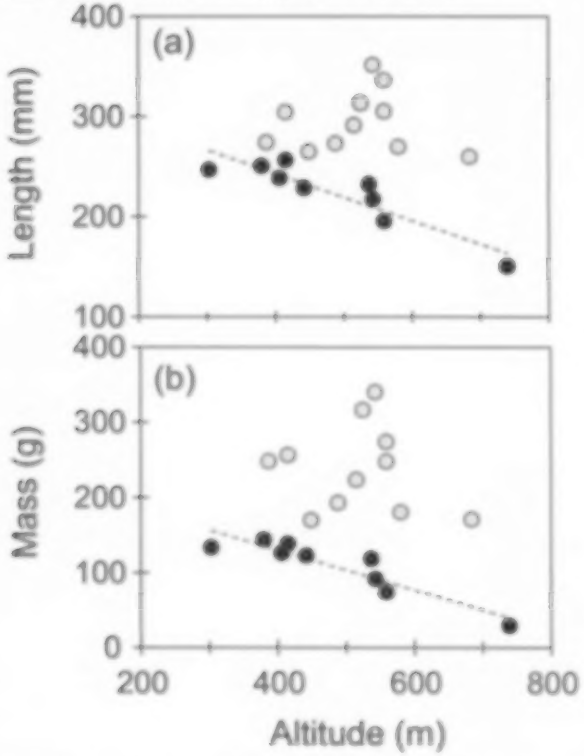
*One-way analysis of variance (ANOVA): $F_{(3,29)} = 21.4$, $P < 0.0001$, $R^2 = 0.72$.

**One-way ANOVA: $F_{(3,29)} = 25.9$, $P < 0.0001$, $R^2 = 0.76$.

the mass of charr in unregulated lakes (Table 1). Likewise, the mean length was 20% and 35% lower in lakes 10–38 and 40–65 years after impoundment, respectively (Table 1).

The size of the charr is negatively related to altitude in regulated lakes but not in unregulated lakes (Fig. 2). We suggest that this is because charr, which are able to utilize both littoral and pelagic food resources (Nilsson 1967; Karlsson and Byström 2005), have a stronger reliance on littoral resources in lakes at high altitudes. Both water column inorganic nutrient concentrations (Rantakari et al. 2004) and the allochthonous organic matter subsidy to the pelagic food web (Jansson et al. 2008) decrease with increasing altitude. Hence, we expect that the relative contribution of the littoral resources increase with altitude in unregulated lakes. The

Fig. 2. Relationship between altitude and (*a*) mean length and (*b*) wet mass of 4+ Arctic charr in unregulated lakes (gray circles) and regulated lakes 10–65 years after impoundment (black circles). Altitude is presented as metres above sea level. Each data point represents the mean for one lake. Linear regressions (broken lines) are statistically significant ($F_{[1,7]} = 34.5$, $P = 0.0006$, $R^2 = 0.83$ for length; $F_{[1,7]} = 39.7$, $P = 0.85$, $R^2 = 0.0004$ for mass, $n = 9$). In unregulated lakes, there is no statistically significant relationship with altitude ($F_{[1,9]} = 0.008$, $P = 0.93$, $R^2 = 0.001$ for length; $F_{[1,9]} = 0.19$, $P = 0.67$, $R^2 = 0.02$ for mass, $n = 11$).



complete loss of a functional littoral zone owing to erosion caused by water level fluctuations in regulated lakes will therefore impose a strong constraint on fish growth at high altitudes, where the nutrient base for the pelagic food web is thin. In contrast, the loss of littoral production in low-altitude reservoirs may at least partly be outweighed by pelagic production. Furthermore, the persistence of abundant littoral resources in unregulated lakes may permit relatively high charr growth rates across the altitude gradient.

There may be several mechanisms behind oligotrophication (decreased ecosystem productivity) in Scandinavian impounded lakes, but they are all most likely a result of the altered hydrological regime. Immigration of anadromous fish does not occur in the studied systems, and other factors such as fishing intensity (Hammar 2005), land use (Moen and Danell 2003), and atmospheric nutrient deposition or acidification (Skjelkvåle et al. 2007) did not change in the studied area along with the construction of the reservoirs. Hence, we suggest the altered hydrological regime as the underlying cause of the observed decline in fish growth. This is due to a decline in ecosystem productivity (oligotrophication, as manifested in decreased charr growth), exposing the littoral zone to wave action and ice erosion. First, when the littoral zone is eroded, littoral nutrient recycling and retention (Wetzel 2001) will be obstructed and nutrients are less efficiently recycled before being lost to deep bottoms and downstream. Second, littoral benthic primary production, which is an important base of the food web of northern low-productive lakes (Karlsson et al. 2009), will largely disappear upon impoundment-induced erosion and fluctuating light regimes. Consequently, the production of benthic invertebrates becomes negligible with negative consequences for fish production and fisheries (Nilsson 1973; Aass 1984; Borgstrøm and Aass 2000). Third, the altered hydrological regime with flooding of the riparian zone delayed from spring (May–June) until after the growing season (October) results in a mismatch between nutrient input and light availability. Hence, much of the nutrients entrained from the catchment in the postimpoundment hydrological regime will be exported downstream before the next growing season starts. This effect will be further amplified at higher altitudes owing to shorter growing season for phytoplankton. Although there are at present no data available that can unequivocally tell us about the relative magnitude of these effects, it is our opinion that the loss of the littoral zone is the key reason for the observed decline in fish growth.

That oligotrophication is the reason behind the decline in fish growth is further corroborated by a whole-lake nutrient enrichment experiment in the regulated Lake Stora Mjölkvattnet (surface area 13 km^2; mean depth 30 m; max depth 96 m). After nutrient enrichment, starting 60 years after impoundment and increasing the summer mean total phosphorus concentration from approximately 4 μg·L^{-1} to approximately 6 μg·L^{-1}, planktonic production was stimulated (Persson et al. 2008) and mean lengths and weights of 4+ Arctic charr increased to levels similar to those in unregulated lakes (Figs. 1*b*, 1*d*) (Rydin et al. 2008). Oligotrophication was first documented in Pacific rivers in North America in the 1960s as an effect of decreased immigration of anadromous fish, thereby reducing input of marine-derived nutrients, and it has been successfully rebuted using nutrient enrichment (Stockner and Macisaac 1996; Hyatt et al. 2004). Likewise, nutrient enrichment has been applied to counteract oligotrophication in North American impounded lakes (Ashley et al. 1997; Pieters et al. 2003).

An alternative explanation to the decline in fish growth rate is that the charr abundance may increase after impoundment, thus resulting in higher resource competition and lower growth rates. Density-dependent growth rates of Arctic charr have previously been reported from an unregulated arctic lake (Amundsen et al. 2007). Hence, a comparison of catch per unit effort across lakes and years in this study would yield valuable information with regard to intraspecific competition and the extent to which it may have influenced the growth rates. Unfortunately, the use of different types of nets (cotton vs. nylon, different mesh sizes, and different lengths) in our data set makes such a comparison of catch per unit effort across all lakes and years difficult and very uncertain. However, results from three unregulated and six regulated lakes during the period 1977–2007 (when nylon nets of similar types were used) suggest that the abundance of Arctic charr is not systematically higher in regulated lakes (Wil-

coxon test, $P = 0.70$), while the standing-stock biomass tended to be lower in regulated lakes in comparison with that in unregulated lakes (Wilcoxon test, $P = 0.07$) (Fig. 3). Furthermore, Aass (1984) reports that after an initial increase in population density following impoundment, the standing stocks decreased both in terms of abundance and biomass. We therefore conclude that the explanation that a long-term increase in abundance following impoundment is not a likely explanation for the reduced fish growth.

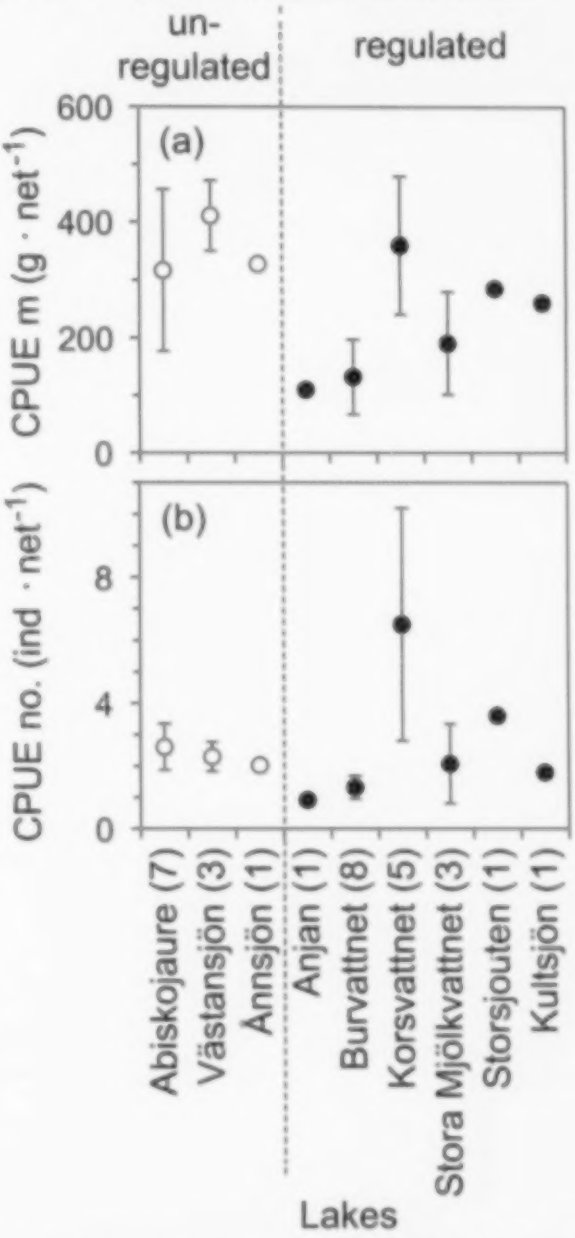
As mentioned before, the lakes in this study are all dominated by Arctic charr, sometimes together with brown trout. Other native or introduced fish species (see Table S1) are of limited importance. In several Scandinavian lakes, the Opossum shrimp, *M. relicta*, had been introduced as a prey organism for planktivorous fish in the 1970s and 1980s (Fürst 1981). In many cases, they have instead become competitors with planktivorous fish for smaller zooplankton (Lasenby et al. 1986). However, in none of the exemplified lakes has *M. relicta* played a key role (Table S1), according to the Institute of Freshwater Research at Drottningholm (Johan Hammar, personal communication, 2009).

Previous studies have shown a short-term inundation effect immediately after impoundment that is manifested as increased nutrient concentrations (Grimard and Jones 1982), increased fish growth and production — "the positive damming-up effect on fish" (Aass 1984; Nilsson 1973; Runnström 1964), and increased greenhouse gas emission (Tremblay et al. 2004). Consistent with these findings, we found a moderate increase of 10% and 21% in charr length and mass, respectively, during the period 1–7 years after impoundment, although the increase was not statistically significant (Fig. 1; Table 1).

The methods both for age determination and sampling varied during the study period and may potentially have affected the results. However, our comparison of age-determination methods clearly showed that scale readings of Arctic charr of ages 3+ to 5+ could be accurately done in these types of lakes (see Appendix S1 for further details), and we are therefore confident that the change in the age-determination method has not introduced any substantial bias. With regard to net types, it is well known that cotton nets are less efficient than monofilamentous nylon nets. However, the mesh sizes used were designed to catch the modal size classes, and largely the modal year classes. Our material does not contradict that contention in the respect that the growth curves fit sufficiently well into each other over time. It should also be noted that the drop in mean size of charr at age 4+ declines gradually irrespective of net type and that in several lakes (e.g., lakes Torrön and Stora Mjölkvattnet) the major drop during the decades following impoundment is documented without major changes in sampling methodology. From the evidence above, we conclude that our findings are not substantially biased by changes in net sampling method.

In the comparison between natural and regulated lakes, long-term trends in the lake material due to, for instance, possible large-scale environmental changes, changed fishing methods and net material used, varying fishing pressure, etc. may possibly lead to bias effects. In the natural lakes and regulated lakes before regulation (Fig. 1) the mean lengths and masses of charr thus tended to decrease (from approximately 310 to 280 mm and from 260 to 200 g, respectively, during the period 1940–2000), even though this long-term trend was not statistically significant ($r = -0.41$, $P = 0.06$; $r = -0.37$, $P = 0.09$). In comparison with the drastic negative effects on charr caused by water regulation, however, this weak trend, whether real or not, is negligible.

Fig. 3. Catch per unit effort of Arctic charr in three unregulated lakes (open symbols) and six regulated lakes (black symbols) during the period 1977–2007. During this period nylon nets with similar mesh sizes and lengths were used. (*a*) Biomass (catch per unit effort CPUE) m, g·net^{-1}), and (*b*) abundance (CPUE no., individuals·net^{-1}). Numbers within parentheses after each lake name indicate sample size (number of years), and error bars show standard deviations.



In conclusion, we demonstrate pronounced negative long-term effects on fish growth, with no sign of recovery, in hydroelectric reservoirs. Hence, hydroelectric power regulation is in conflict with the sustainable management of fish populations that are of great value for commercial fishing as well as game fishing. Although the long-term decline in fish growth is clearly demonstrated, the results give rise to new research questions. Hence, there is a need to further elucidate and quantify the underlying mechanisms behind the pattern, to investigate whether there are changes in growth, reproduction, and population dynamics of Arctic charr and other co-occurring fish species (especially brown trout) and taking all age classes into account. Finally, similar ecosystem damages may be a general concern also outside Scandinavia in reservoirs with large water level fluctuations.

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References

Aass, P. 1984. Management and utilization of Arctic charr in Norwegian hydroelectric reservoirs. *In* Biology of the Arctic Charr: Proceedings of the International Symposium on Arctic Charr. *Edited by* L. Johnson and B. Burns. University of Manitoba Press, Winnipeg, Man. pp. 277–291.

Amundsen, P.A., Knudsen, R., and Klemetsen, A. 2007. Intraspecific competition and density dependence of food consumption and growth in Arctic charr. J. Anim. Ecol. **76**(1): 149–158. doi:10.1111/j.1365-2656.2006.01179.x. PMID:17184363.

Ashley, K., Thompson, L.C., Lasenby, D.C., McEachern, L., Smokorowski, K.E., and Sebastian, D. 1997. Restoration of an interior lake ecosystem. The Kootenay Lake fertilization experiment. Water Qual. Res. J. Canada, **32**: 295–323.

Borgstrøm, R., and Aass, P. 2000. Vassdragsregulering. *In* Fisk i ferskvann: et samspill mellom bestander, miljø og forvaltning. *Edited by* R. Borgstrøm and L. Hansen. Landbruksforlaget, Oslo, Norway. pp. 216–246. [In Norwegian.]

Chao, B.F., Wu, Y.H., and Li, Y.S. 2008. Impact of artificial reservoir water impoundment on global sea level. Science (Washington, D. C.), **320**(5873): 212–214. doi:10.1126/science.1154580. PMID: 18339903.

Conover, W.J. 1980. Practical nonparametric statistics. 2nd ed. John Wiley, New York.

European Committee for Standardization. 2005. Water quality — sampling of fish with multi-mesh gill nets, EN 14757:2005. European Committee for Standardization, Brussels.

Filipsson, O. 1967. Åldersbestämning av röding med hjälp av otoliter. Report No. 5. Swedish Board of Fisheries, Institute of Freshwater Research, Drottningholm, Sweden. pp. 1–23. [In Swedish.]

Fürst, M. 1981. Results of introductions of new fish food organisms into Swedish lakes. Rep. Inst. Freshw. Res. Drottningholm, **59**: 33–47.

Grimard, Y., and Jones, H.G. 1982. Trophic upsurge in new reservoirs: a model for total phosphorus concentration. Can. J. Fish. Aquat. Sci. **39**(11): 1473–1483. doi:10.1139/f82-199.

Hammar, J. 2005. Röding, öring och överfisket i fjällen. Biologiska förutsättningar och strategier för ett uthålligt nyttjande av fiskbestånd i fjällvatten. Report, Swedish Board of Fisheries, Institute of Freshwater Research, Drottningholm, Sweden. [In Swedish.]

Hammar, J., and Filipsson, O. 1985. Ecological testfishing with the Lundgren gillnets of multiple mesh size: the Drottningholm technique modified for Newfoundland Arctic char populations. Rep. Inst. Freshw. Res. Drottningholm, **62**: 12–35.

Humborg, C., Ittekkot, V., Cociasu, A., and VonBodungen, B. 1997. Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. Nature (Lond.), **386**(6623): 385–388. doi:10.1038/386385a0.

Hyatt, K.D., McQueen, D.J., Shortreed, K.S., and Rankin, D.P. 2004. Sockeye salmon (*Oncorhynchus nerka*) nursery lake fertilization: review and summary of results. Environ. Rev. **12**(3): 133–162. doi:10.1139/a04-008.

Jansson, M., Hickler, T., Jonsson, A., and Karlsson, J. 2008. Links between terrestrial primary production and bacterial production and respiration in lakes in a climate gradient in subarctic Sweden. Ecosystems (N. Y.), **11**(3): 367–376. doi:10.1007/s10021-008-9127-2.

Karlsson, J., and Byström, P. 2005. Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. Limnol. Oceanogr. **50**(2): 538–543. doi:10.4319/lo.2005.50.2.0538.

Karlsson, J., Bystrom, P., Ask, J., Ask, P., Persson, L., and Jansson, M. 2009. Light limitation of nutrient-poor lake ecosystems. Nature (Lond.), **460**(7254): 506–509. doi:10.1038/nature08179. PMID: 19626113.

Lasenby, D.C., Northcote, T.G., and Fürst, M. 1986. Theory, practice, and effects of *Mysis relicta* introductions to North American and Scandinavian lakes. Can. J. Fish. Aquat. Sci. **43**(6): 1277–1284. doi:10.1139/f86-158.

Moen, J., and Danell, Ö. 2003. Reindeer in the Swedish mountains: an assessment of grazing impacts. Ambio, **32**(6): 397–402. PMID: 14627368.

Nilsson, N.-A. 1967. Interactive segregation between fish species. *In* The biological basis of freshwater fish production. *Edited by* S.D. Gerking. Blackwell Scientific, Oxford, UK. pp. 295–313.

Nilsson, N.-A. 1973. Biological effects of water-power exploitation in Sweden, and means of compensation for damage. *In* Congrès international des grands barrages, Madrid, Spain, 11–15 June 1973. International Commission on Large Dams, Paris, France. pp. 923–940.

Nilsson, C., Reidy, C.A., Dynesius, M., and Revenga, C. 2005. Fragmentation and flow regulation of the world's large river systems. Science (Washington, D.C.), **308**(5720): 405–408. doi:10.1126/science.1107887. PMID:15831757.

Persson, J., Vrede, T., and Holmgren, S.K. 2008. Responses in zooplankton populations to food quality and quantity changes after whole lake nutrient enrichment of an oligotrophicated sub-alpine reservoir. Aquat. Sci. **70**(2): 142–155. doi:10.1007/s00027-007-7013-1.

Pieters, R., Harris, S., Thompson, L.C., Vidmanic, L., Rousshorne, M., Lawrence, G., Stockner, J.G., Andrusak, H., Ashley, K.I., Lindsay, B., Hall, K., and Lombard, D. 2003. Restoration of Kokanee salmon in the Arrow lakes Reservoir, British Columbia: preliminary results of a fertilization experiment. *In* Nutrients in salmonid ecosystems: sustaining production and biodiversity. *Edited by* J.G. Stockner. American Fisheries Society, Bethesda, Md. pp. 177–196.

Poff, N.L., Olden, J.D., Merritt, D.M., and Pepin, D.M. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. Proc. Natl. Acad. Sci. U.S.A. **104**(14): 5732–5737. doi:10.1073/pnas.0609812104. PMID:17360379.

Rantakari, M., Kortelainen, P., Vuorenmaa, J., Mannio, J., and Forsius, M. 2004. Finnish lake survey: the role of catchment attributes in determining nitrogen, phosphorus, and organic carbon concentrations. Water Air Soil Pollut. Focus, **4**(2/3): 683–699. doi:10.1023/B:WAFO.0000028387.61261.96.

Runnström, S. 1955. Changes in fish production in impounded lakes. Verh. Int. Ver. Theor. Angew. Limnol. **12**: 176–182.

Runnström, S. 1964. Effects of impoundment on the growth of *Salmo trutta* and *Salvelinus alpinus* in Lake Ransaren (Swedish Lappland). Verh. Int. Ver. Theor. Angew. Limnol. **15**: 453–461.

Rydin, E., Vrede, T., Persson, J., Holmgren, S., Jansson, M., Tranvik, L., and Milbrink, G. 2008. Compensatory nutrient enrichment in an oligotrophicated mountain reservoir — effects and fate of added nutrients. Aquat. Sci. **70**(3): 323–336. doi:10.1007/s00027-008-8061-x.

Skjelkvåle, B.-L., Borg, H., Hindar, A., and Wilander, A. 2007. Large scale patterns of chemical recovery in lakes in Norway and Sweden: importance of seasalt episodes and changes in dissolved organic carbon. Appl. Geochem. **22**(6): 1174–1180. doi:10.1016/j.apgeochem.2007.03.040.

St. Louis, V.L., Kelly, C.A., Duchemin, E., Rudd, J.W.M., and Rosenberg, D.M. 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. Bioscience, **50**(9): 766–775. doi:10.1641/0006-3568(2000)050[0766:RSASOG]2.0.CO;2.

Stockner, J.G., and Macisaac, E.A. 1996. British Columbia lake enrichment programme: two decades of habitat enhancement for sockeye salmon. Regul. Rivers, **12**(4–5): 547–561. doi:10.1002/(SICI)1099-1646(199607)12:4/5<547::AID-RRR407>3.0.CO;2-M.

Stockner, J.G., Rydin, E., and Hyenstrand, P. 2000. Cultural oligotrophication: causes and consequences for fisheries resources. Fisheries (Bethesda, Md.), **25**(5): 7–14. doi:10.1577/1548-8446(2000)025<0007:CO>2.0.CO;2.

Tremblay, A., Lambert, M., and Gagnon, L. 2004. Do hydroelectric reservoirs emit greenhouse gases? Environ. Manage. **33**(S1): S509–S517. doi:10.1007/s00267-003-9158-6.

Wetzel, R.G. 2001. Limnology. Academic Press, San Diego, Calif.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.

Fishing-gear threat to right whales (*Eubalaena glacialis*) in Canadian waters and the risk of lethal entanglement

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Abstract: Commercial fishing gear can potentially entangle any whale, and this is especially true for the endangered North Atlantic right whale (*Eubalaena glacialis*), for which entanglement is second only to vessel strike as being responsible for documented right whale deaths. We use right whale survey data and Canadian fishing-gear deployment data to estimate the relative threat of gear entanglement in a Scotia–Fundy study area and the relative risk of lethal entanglement in the Bay of Fundy and on Roseway Basin, Scotian Shelf, where Critical Habitat has been legislated. We focus on groundfish and pelagic hook-and-line; groundfish gillnet; and crab-, hagfish-, and inshore and offshore lobster-trap gear. Our analyses demonstrate that groundfish hook-and-line gear poses the greatest threat to right whales among the seven gear types analysed during the summer-resident period in Critical Habitat and that gear from the lobster fisheries poses the greatest threat during the spring and autumn periods when whales are migrating to and from Critical Habitat. We suggest that area-specific seasonal closures of some fisheries would reduce threat and risk to whales without unduly compromising fishing interests.

Résumé : Toutes les baleines peuvent potentiellement s'empêtrer dans les engins de pêche commerciale et cela est particulièrement le cas de la baleine franche du Nord (*Eubalaena glacialis*) chez qui l'enchevêtrement est la seconde cause connue de mortalité après les chocs avec les navires. Nous utilisons les données d'inventaire des baleines franches du Nord et les données canadiennes sur le déploiement des engins de pêche pour estimer la menace relative de l'enchevêtrement dans les engins de pêche dans une zone d'étude de Scotia–Fundy, ainsi que le risque relatif d'enchevêtrement létal dans la baie de Fundy et le bassin de Roseway sur la plateforme néo-écossaise où un habitat critique a été désigné par législation. Nous nous intéressons à la pêche à la ligne et à l'hameçon des poissons benthiques et pélagiques, à la pêche au filet maillant des poissons de fond et à la pêche au casier de crabes, de myxines et de homards, tant au large que près des côtes. Nos analyses démontrent que les engins de pêche à la ligne et aux hameçons des poissons de fond représentent la menace la plus importante pour les baleines franches du Nord parmi les sept types d'engins analysés durant la période de résidence d'été des baleines dans l'habitat critique; les engins de pêche aux homards posent la menace la plus grande durant les périodes de printemps et d'automne quand les baleines migrent vers l'habitat critique ou en ressortent. Nous proposons des fermetures saisonnières de certaines pêches commerciales dans des zones précises, ce que réduirait la menace et le risque pour les baleines, sans compromettre outre mesure les intérêts de la pêche commerciale.

[Traduit par la Rédaction]

Introduction

Any cetacean can potentially become entangled in fishing gear, and entanglements are a major source of anthropogenic mortality among whales (Reeves et al. 2003). Although small whales are less likely to free themselves from an entanglement and thus drown, an entangled baleen whale is capable of dragging the gear for extended periods (Clapham et al. 1999). Gear entanglements are not necessarily lethal for large whales, and many right whales appear to shed gear or self-disentangle with no chronic effect (Johnson et al. 2007).

The frequency of reported entanglements varies among whale species and regions, and coastal species appear especially vulnerable. This is particularly true for the humpback whale (*Megaptera novaeangliae*) and the North Atlantic right whale (*Eubalaena glacialis*; Rosenbaum et al. 2000; hereafter referred to as right whale) as documented by Kraus (1990) and Lien (1994), wherein the entanglements are associated with various kinds of fishing gear that include longlines (hook-and-line), drift nets, traps or pots, and gillnets, etc. Johnson et al. (2005) report that 89% (32/36) of documented entanglements of humpback and right whales in the north-

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west Atlantic are attributable to trap (pot) and gillnet gear, when gear was identifiable. Between 80% and 90% of large cetacean entanglements in coastal Newfoundland and Labrador are attributable to cod traps and groundfish gillnets (Lien 1994).

Of all documented right whale deaths attributed to human activities, death from entanglement in fishing gear is second only to death from vessel strike. Over the period 1970 through 1999, Knowlton and Kraus (2001) report that 6.7% of documented right whale mortalities and 55% of serious injuries were due to entanglement. The percentage of right whale deaths attributable to entanglement increases to 13% when estimates are based only on those whales necropsied over the period 1970 through 2002 (Moore et al. 2004). Approximately 73% of all photographically identified right whales show scarring that is consistent with at least one entanglement (Knowlton et al. 2008), and nearly every year an entangled whale either dies or disappears from the photographic-identification record (Johnson et al. 2007). Moore et al. (2006) estimate that between 49 and 136 right whales are entangled annually, and they conclude that the number of lethal entanglements is likely underestimated. As preventing the deaths of two female right whales per year has a measureable influence on population growth rate (Fujiwara and Caswell 2001), measuring the threat of fishing-gear entanglement provides the information required to mitigate negative influences on the population. As there is virtually no information on the nature of whale and fishing gear "interaction" and exactly how a whale becomes entangled, the simplest method to reduce the risk of entanglement is to reduce the amount and extent of fishing gear in those habitats and at those times where and when the whales are expected to be present (Kraus et al. 2005).

The threat to large whales of fishing-gear entanglements from commercial fisheries is being addressed in the United States of America (USA) via the imposition of selective area closures and gear modifications (e.g., National Oceanic and Atmospheric Administration 2007*a*, 2007*b*). Gear modifications include the use of weak links at buoys and the use of sinking groundlines, where the latter are required at various times throughout the range of right whales in USA waters (National Oceanic and Atmospheric Administration 2007*b*). Such gear modifications are designed to reduce the probability of entanglement and increase the likelihood of self-disentanglement. Fishing closures and the reduction of lines in the water column most simply reduce mortality via reduced probability of a gear encounter and thus lethal entanglement. In contrast with the USA, there have been no mandatory modifications of fishing practices in Canadian waters to protect right whales where and when they migrate to and from the Bay of Fundy and Roseway Basin feeding habitats, where the whales aggregate during the July through October period.

Right whale conservation initiatives in Canadian waters have included the designation of Right Whale Conservation Area(s) (Brown et al. 1995) and Right Whale Critical Habitat (Brown et al. 2009; as illustrated in Fig. 1) and modified vessel navigation in the Bay of Fundy and Roseway Basin regions (Vanderlaan et al. 2008; Vanderlaan and Taggart 2009). Although the conservation areas served to warn mariners of the presence of right whales and were associated with recommendations for voluntary actions to minimize the probability of a vessel strike, they did not provide regulations, recommendations, or guidelines for fishing practices or sanctions to minimize the probability of an entanglement. In effect, the conservation areas no longer exist, as they have been superseded by Critical Habitat areas (Brown et al. 2009). According to the Canadian Species at Risk Act (SARA: Species at Risk Act 2002), activities within a Critical Habitat that would destroy the habitat are prohibited, though such prohibitions do not explicitly address fishing practices. Any modifications to contemporary fishing practices for the protection of right whales in Canadian waters, either through fishing closures or gear modifications, will require quantitative estimates of the threat or risk to the whales from fishing gear, as well as where and when entanglements are most likely to occur.

Here we quantify the relative threat of Canadian fishing-gear entanglement to right whales in the Scotia–Fundy region (Fig. 1). We then focus on estimating the risk of lethal entanglement in regions defined as Critical Habitat under SARA (Brown et al. 2009): the Grand Manan Basin region in the Bay of Fundy and the Roseway Basin region on the southwest Scotian Shelf (Fig. 1). We focus on six different fisheries and their associated gear: the crab-, hagfish-, and offshore lobster-trap (pot) fisheries; the groundfish gillnet and hook-and-line fisheries; and the pelagic hook-and-line fishery. We also address a seventh, the inshore lobster-trap fishery, though the data are much more limited in relation to the above six fisheries. We use the results to identify possible management methods that could be used to minimize the threat and risk of lethal entanglements in right whale habitat without unduly compromising fishing interests.

Material and methods

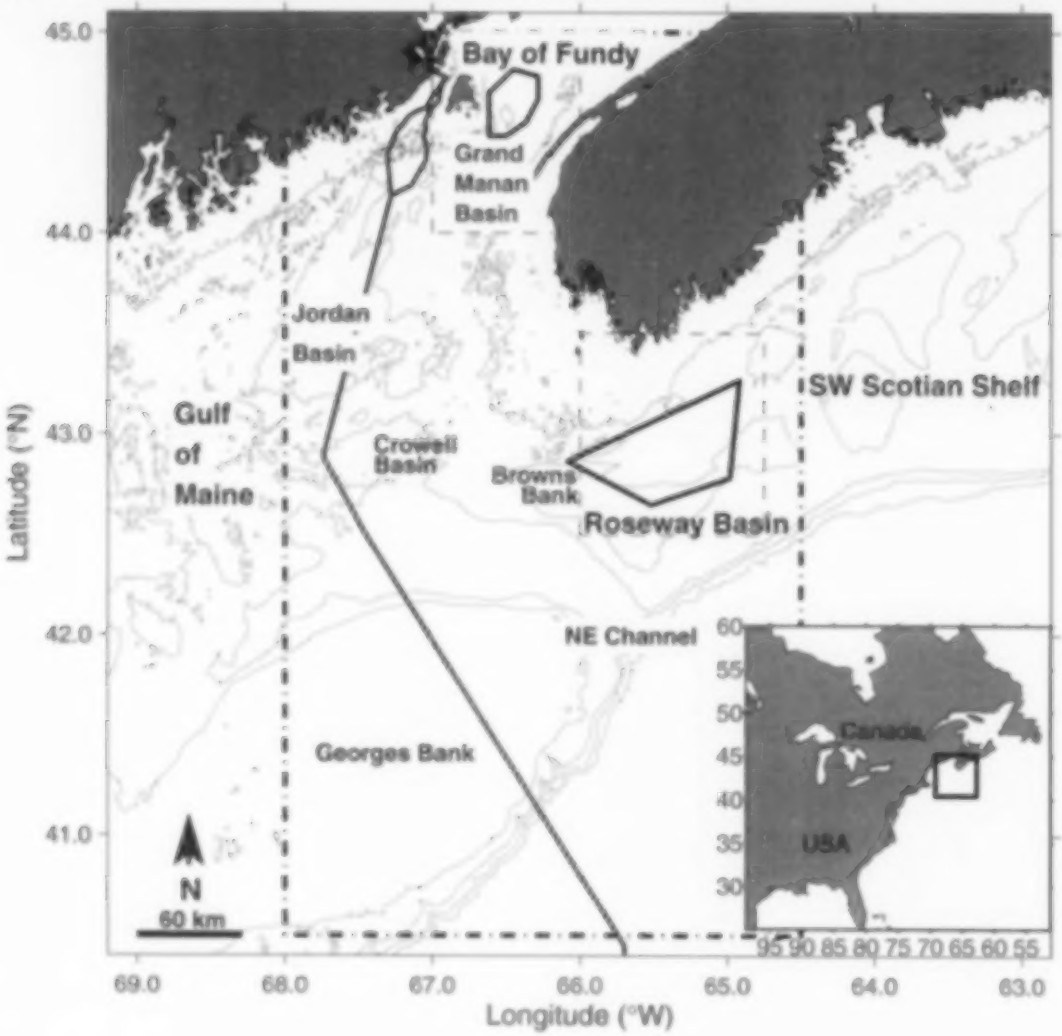
Overview

We quantitatively addressed (*i*) the temporal coincidence of fishing gear and right whales in the Scotia–Fundy study area, (*ii*) the threat of right-whale entanglement in the Scotia–Fundy study area during the spring-immigration period, the summer-resident (feeding) period, and the autumn-emigration period, and (*iii*) the risk of lethal right-whale entanglement in the Bay of Fundy and Roseway Basin study areas (Fig. 1). The Scotia–Fundy study area was defined by 40.5°N through 45.0°N latitude and 64.5°W through 68.0°W longitude (area ≈ 194 500 km^2). The smaller study areas (Fundy and Roseway) were delineated by right-whale survey and whale-sighting data (sightings per unit effort, SPUE) that were used for similar purposes when addressing the risk of lethal vessel strikes (Vanderlaan et al. 2008).

Whale and fishing gear data

Quality controlled right whale SPUE data for the period 1979 through 2007 were provided for each of the Fundy and Roseway study areas by the North Atlantic Right Whale Consortium (North Atlantic Right Whale Consortium 2008). An overview of the right-whale surveys are provided in Brown et al. (2007), and the most salient features are provided in Vanderlaan et al. (2008). The aggregate 1979–2007 SPUE data were resolved over 20 × 20 cell (Fundy; area ≈ 8643 km^2) and 25 × 20 cell (Roseway; area ≈ 11 319 km^2)

Fig. 1. Bathymetric (100, 200, 500, and 1000 m isobaths) chart illustrating the location (inset) of the Scotia–Fundy study area (black dot-dash rectangle), the Bay of Fundy and Roseway Basin study areas (black dash rectangles) and associated right whale Critical Habitat (black solid-line polygons), and the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (grey solid line).



grids, where each grid-cell was defined by 3′N latitude and 3′W longitude (5.6 km N–S and ~3.9 km E–W for Fundy and ~4.1 km E–W for Roseway). This was the limiting resolution used for all spatial analyses below except when noted. The total Canadian open-waters area in the Scotia–Fundy study area is ≈87 800 km^2 and those for the Fundy and Roseway study areas are ≈8200 km^2 and ≈11 200 km^2, respectively, representing 9% and 13% of Canadian open waters in the Scotia–Fundy study area.

The fishing-gear deployment data for the six fisheries above were derived from two statistical databases detailing commercial-fishery landings that are maintained by Fisheries and Oceans Canada (DFO). The databases detail information for all fishing trips where a landing is reported within the DFO Maritimes region. Our aggregate gear-specific data for the period 1999 through 2007 were extracted from the Zonal Interchange File Format (ZIFF) database for 1999 through 2001 and from the Marine Fish (MARFIS) database for 2002 through 2007. Each database, along with details on gear configuration, is described in Johnston et al. (2007). The data contain a variety of information pertaining to date, trip, gear type, set location, amount of gear used in a set, and species landed, etc. All of our analyses involving fishing gear are based on date and location of each set, where each set is quantified by the amount of gear deployed, G, (i.e., number of hooks, nets or panels, traps or pots, deployed at a set location and time). We also use only the number of sets deployed within a fishery regardless of the amount of gear used within a set. Species-specific landings data are used for interpretive considerations in the Discussion.

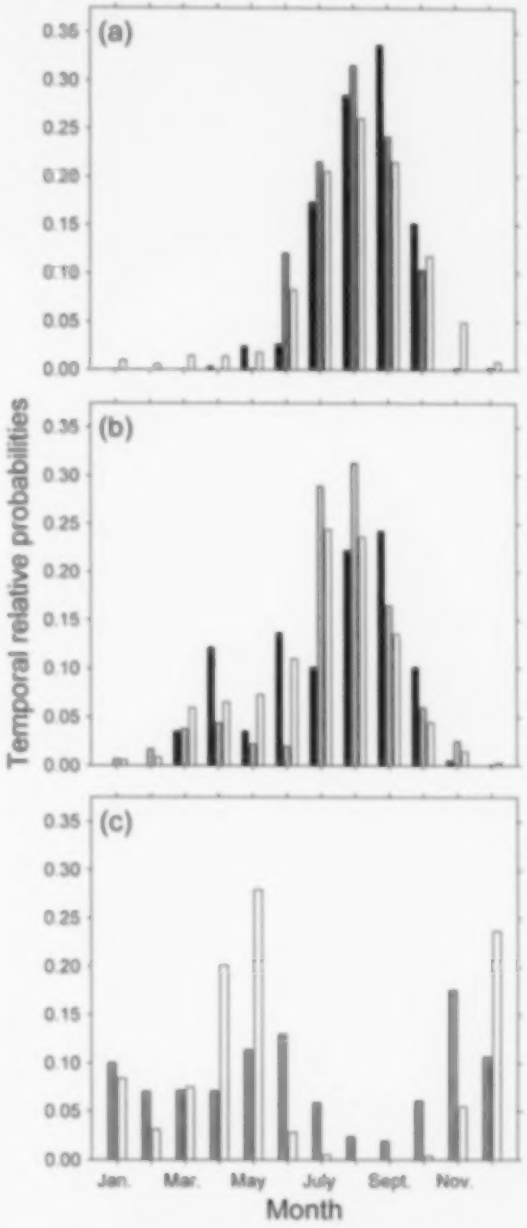
The resolution of the inshore lobster-fishery data is considerably different from that associated with the six fisheries

above in that the data were provided in aggregate across "logbook reporting areas", with a spatial resolution varying from 100 to 340 km^2. Further, the number of sets deployed is not reported for the inshore-lobster fishery, and as the only measure reported is the number of traps hauled, we assume it is equivalent to G. We cannot quantify risk of entanglement, as the low and varying spatial and temporal resolution prevents us from achieving the same relatively high resolution associated with the other fisheries. Thus, the inshore lobster-gear data and analyses are treated separately from the other fisheries. There are very likely reporting biases associated with the inshore logbook data that cannot be assessed. To minimize potential bias, we selected only those years (2005–2008 within the 1998–2010 series) with the most extensive and relatively constant spatial and temporal coverage. The number of logbooks reporting traps hauled in 1998 was 67, and this rose systematically to 592 in 2004. Over the period 2005–2008, the mean and standard deviation of logbooks reporting hauls was 801 and 12, respectively. There were 568 logbooks reporting hauls in 2009, and this declined to 214 in 2010. A portion (~14%) of inshore-lobster fishers reports haul data as part of the offshore-lobster fishery, and we have used those data as part of the offshore fishery.

The aggregate gear-specific data (except inshore lobster) were spatially resolved over a 90 × 70 cell grid covering the Scotia–Fundy study area that also encompasses the smaller Fundy and Roseway study areas (Fig. 1), and each cell was defined as above for the right whale data. We also examined fishing-gear threat in the Scotia–Fundy study area using aggregate data over three seasonal periods relevant to the whales: (1) May through June — spring immigration, (2) July through October — summer resident, and (3) November through December — autumn emigration. These periods were based on the seasonal migration and aggregation patterns for right whales in Canadian waters (Winn et al. 1986; Murison and Gaskin 1989; Gaskin 1991; see also Fig. 2). The relative risk of a lethal entanglement in the Fundy and Roseway study areas was estimated for the summer-resident period only.

The ZIFF and MARFIS databases include incomplete records (null observations) within and among the various fisheries. For example, the number of nets in a groundfish gillnet set (deployment) was not recorded after 2004. Some of the data were considered to be erroneous (statistical outliers) or unsubstantiated; e.g., hooks per set were sometimes >10 000 in the groundfish hook-and-line fishery and are considered incorrect (Johnston et al. 2007). Such data were initially nullified. To replace a null datum within a set, a k-nearest neighbour imputation (Jönsson and Wohlin 2004) was used, as the numbers of hooks, traps, etc. were spatially autocorrelated and their distributions were skewed. To complete the imputations, the data were classified into two subsets: the first where the number of hooks, traps, etc. was available and the second that contained the null observations. For each observation in the "null" subset, the Euclidean distance to each observation in the "available" subset was calculated. The median value for the number of hooks, traps, etc. of the k = 25 neighbours nearest the null observation was then used to replace the null observation.

Fig. 2. Histograms of the monthly temporal relative probabilities of observing (*a*) right whales (P_{rel}(Whale), black bars) and gear (P_{rel}(Gear)) for each of (*a*) pelagic (grey bars) and groundfish (open bars) hook-and-line fisheries, (*b*) hagfish- (black bars) and crab- (grey bars) trap fisheries and the groundfish-gillnet fishery (open bars), and (*c*) the offshore- (grey bars) and inshore- (open bars) lobster fisheries in the Scotia–Fundy study area.



Temporal coincidence of fishing gear and right whales

To assess the temporal coincidence between whales and the amount of gear deployed across the entire Scotia–Fundy study area, and thus the temporal variation in threat of entanglement for right whales, the temporal relative probabilities

of the amount of gear being deployed and of observing right whales in the Scotia–Fundy domain were calculated for each month. Fishing-gear data for all fisheries, including inshore lobster, were aggregated by month across the years 2005 through 2008 (4 years), because of the limitations of the inshore lobster-gear data. The number of traps set in the offshore lobster fishery was only reported prior to 2002, and for this reason, and to be consistent with the other fisheries, the temporal relative probability of offshore lobster gear being deployed was estimated using the 1999 though 2001 data (3 years).

We assumed that the above temporal aggregations of fishing-gear data can be used to provide the best estimate of the relative probability (0,1) of observing a given type of fishing gear, P_{rel}(Gear), over a given month (t) in the Scotia–Fundy study area by using the number (G) of hooks, nets, or traps, etc. deployed over the month and was calculated as

$$(1) \quad P_{rel}(\text{Gear})_t = \frac{G_t}{\sum_{t=1}^{12} G_t}$$

Similarly, and as in Vanderlaan et al. (2008, 2009), we assumed that the 1979–2007 aggregate SPUE estimates can be used to provide the best estimate of the relative probability of observing a right whale, P_{rel}(Whale), over a given month (t) in the Scotia–Fundy study area by using SPUE in a given month, as in eq. 1 above.

To quantify the temporal coincidence of P_{rel}(Whale) with P_{rel}(Gear) for each fishery in the Scotia–Fundy study area across months, we used a sum of squares (SSW) statistic defined as

$$(2) \quad \text{SSW} = \sum_{t=1}^{12} \left[P_{rel}(\text{Gear})_t - P_{rel}(\text{Whale})_t\right]^2$$

where large estimates of SSW represent large differences in the temporal coincidence of the relative probability estimates. Seasonal cross-correlations using monthly lags between P_{rel}(Whale)$_t$ and P_{rel}(Gear)$_t$ were also used as a measure of the temporal similarity between the two P_{rel} estimates for each fishery in the Scotia–Fundy study area.

Threat of fishing-gear entanglement in the Scotia–Fundy study area

We assumed that the aggregate fishing-gear data provide the best estimates of P_{rel}(Gear) at a 3′ spatial resolution across grid-cells (i) for the amount of each type of fishing gear deployed, which was calculated as

$$(3) \quad P_{rel}(\text{Gear})_i = \frac{G_i}{\sum_{i=1}^{n} G_i}$$

To quantify the threat of a fishing-gear entanglement, we weighted the G_i estimate by depth to the seabed (local bathymetry at each grid-cell centroid), as the end-buoy lines associated with each set extend throughout the water column and present an additional threat of entanglement. For all grid-cells where depth was >200 m, a weight of 200 was used, as right whales generally tend to dive to a depth less than this (Winn et al. 1995; Nowacek et al. 2001, 2004).

The relative threat of an entanglement in fishing gear in a grid-cell, relative to all other cells in a domain of n cells, was calculated as

$$(4) \quad P_{rel}(\text{Threat})_i = \frac{G_i \times W_{i\forall \le 200}}{\sum_{i=1}^{n} G_i \times W_{i\forall \le 200}}$$

where W_i is the depth (m) to the seabed in a grid-cell. To compare relative threat measures across different periods (immigration, summer-resident, and emigration), eq. 4 was modified to

$$(5) \quad P_{rel}(\text{Threat})_i = \frac{G_i \times W_{i\forall \le 200}}{\sum_{i=1}^{m} G_i \times W_{i\forall \le 200}}$$

where $m = (n_{\text{immigration}} + n_{\text{resident}} + n_{\text{emigration}})$ or $m = (n_{\text{immigration}} + n_{\text{emigration}})$ as required.

For the Scotia–Fundy study area, the relative threat of a fishing-gear entanglement (eq. 4) was estimated for the summer-resident period, and the standardized relative threat of entanglement (eq. 5) was estimated for comparative purposes between the right whale spring-immigration and autumn-emigration periods.

Risk of fishing-gear entanglement in Bay of Fundy and Roseway Basin

We use the formal definition of risk (Kaplan and Garrick 1981) that in this context is the intersection of the probability of a whale encountering gear and the probability of a lethal injury given an encounter has occurred: P(Lethal | Encounter). We used an approach similar to that of Wiley et al. (2003) and Vanderlaan et al. (2008) to estimate the relative probability that fishing gear and a whale will occupy (encounter each other in) a given space (grid-cell). As in Vanderlaan et al. (2008, 2009), we assumed that the 1979–2007 aggregate SPUE estimates provide the best estimate of the relative probability, at 3′ resolution, that a whale occupies a grid-cell (SPUE$_i$) relative to cells (i) in a domain and was calculated in a manner similar to eq. 4 above:

$$(6) \quad P_{rel}(\text{Whale})_i = \frac{\text{SPUE}_i}{\sum_{i=1}^{n} \text{SPUE}_i}$$

Using eq. 4 and eq. 6 above, the relative probability that fishing gear and a whale will occupy a given grid-cell was then calculated as

$$(7) \quad P_{rel}(\text{Encounter})_i = \frac{P_{rel}(\text{Whale})_i \times P_{rel}(\text{Threat})_i}{\sum_{i=1}^{m} P_{rel}(\text{Whale})_i \times P_{rel}(\text{Threat})_i}$$

where P_{rel}(Encounter)$_i$ is normalized such that the sum across the grid is equal to one.

Probability of lethal entanglement

To estimate the lethality of an entanglement, we first estimated the total, population-wide (entire range) number of

gear entanglements over the period of 1986 through June 2005 using right whale population abundance (Kraus and Rolland 2007), entanglement rate (Knowlton et al. 2008), and entanglement reports (Moore et al. 2006). We then estimated P(Lethal|Encounter) using the total number of deaths (observed, presumed, or expected; Moore et al. 2006) resulting from an entanglement divided by the total number of entanglements. The errors associated with the probability estimates were calculated using conventional propagation of error.

The relative risk (RR) of a lethal entanglement in the Bay of Fundy and Roseway Basin study areas during the right whale summer-feeding period was estimated using the relative probability of an encounter and the probability that the encounter is lethal

$$\text{(8)} \qquad \text{RR}_t = P_{\text{rel}}(\text{Encounter})_t \times P(\text{Lethal}|\text{Encounter})_t$$

Results

Estimating the lethality of an entanglement

The Knowlton et al. (2008) scarring-study data, based only on "adequately photographed" whales in consecutive years, allowed us to estimate an average annual entanglement rate of 0.28 ± 0.11 over a 23-year period (1982 through 2004). This estimate corresponds to annual estimate of $e_1 = 97 \pm 39$ individual entanglements sufficient to cause scarring, but not death, based on a whale population size of 350 individuals (Kraus and Rolland 2007). Moore et al. (2006) reports $e_2 = 23$ lethal entanglements (observed, presumed, or expected) over a period $t = 19.5$ years (1986 through June 2005). The above estimates allowed us to estimate the probability of a lethal entanglement given an encounter as

$$\text{(9)} \qquad P(\text{Lethal}|\text{Encounter}) = \frac{e_2}{e_1 \times t + e_2} = 0.012 \pm 0.0047$$

i.e., approximately a 1% chance of a right whale dying as the result of a fishing-gear entanglement.

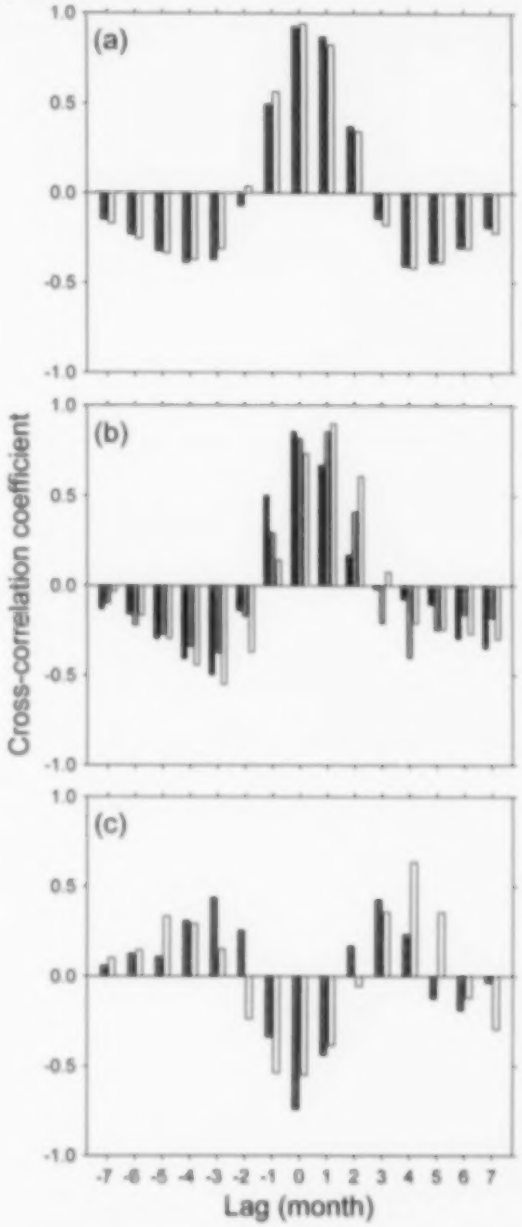
The paucity of data concerning gear-specific entanglements among right whales (Johnson et al. 2005) forces us to assume that all fishing gear is equally likely to be lethal if encountered by a right whale. The consequences of this assumption are addressed in detail in the Discussion.

Temporal coincidence of fishing gear and right whales

The greatest (>0.15) temporal relative probabilities of observing a right whale in the Scotia–Fundy study area occur in July through September, a period that corresponds to the greatest relative probabilities of pelagic and groundfish hook-and-line gear being deployed (Fig. 2*a*), and similarly for hagfish-trap and crab-trap and groundfish-gillnet gear (Fig. 2*b*). Conversely, while whale probabilities are greatest during the July–September period, the probabilities of inshore and offshore lobster gear being deployed over the same period are at their lowest and zero for inshore gear during August and September when the fishery is closed (Fig. 2*c*).

The fishery with the lowest SSW statistic, and thus the greatest temporal coincidence with the presence of right whales over the entire Scotia–Fundy study area, is the pelagic hook-and-line fishery (SSW = 2.34×10^{-2}), closely followed by the groundfish hook-and-line fishery (SSW = 2.35×10^{-2}). These

Fig. 3. Temporal cross-correlation estimates at 1-month lags between aggregate (1979 through 2007) right whale SPUE estimates and aggregate (2004 through 2007 except offshore lobster; 1999 through 2001) amounts of fishing gear deployed (G_t) for each of the (*a*) pelagic (grey bars) and groundfish hook-and-line (open bars) fisheries; (*b*) hagfish-trap (black bars), crab-trap (grey bars), and groundfish-gillnet (open bars) fisheries; and (*c*) offshore (grey bars) and inshore (open bars) lobster-trap fisheries, in the Scotia–Fundy study area.



two fisheries also have the highest temporal cross-correlation near one at zero lag, each decreasing to negative cross-correlations at –4 and +4 month lags (Fig. 3*a*). Thus, the

groundfish and pelagic hook-and-line fisheries are temporally coincident and near-perfectly in phase with the right whales in the Scotia–Fundy area. The crab- and hagfish-trap and groundfish-gillnet fisheries have greater SSW statistics of 5.59×10^{-2}, 4.79×10^{-2}, and 7.62×10^{-2}, respectively, though their temporal cross-correlations with right whales at zero lag are relatively high, with lags decreasing to negative at ≤ -3 and $\geq +4$ months (Fig. 3*b*), indicating moderate temporal coincidence with right whales. Relative to the other fisheries above, the lobster fisheries have the highest SSW statistics of 4.20×10^{-1} for the inshore and 2.76×10^{-1} for the offshore. In contrast with the other fisheries, the lobster fisheries are negatively cross-correlated with the whales at zero lag and increase toward positive values at lags of ≤ -3 to $\geq +3$ months, indicating that these fisheries are out of phase with the presence of right whales in the Scotia–Fundy study area (Fig. 3*c*). The relatively large SSW statistics and cross-correlation analyses indicate temporal separation between the deployment and hauling of lobster gear and the presence of right whales. The SSW statistic for the inshore-lobster fishery is not only the highest among all fisheries, it is fivefold greater than any other fishery with the exception of the offshore-lobster fishery. The SSW statistics for the inshore and offshore fisheries are, respectively, also 12- and 18-fold greater than each of the hook-and-line fisheries, indicating temporal separation between the lobster gear, both inshore and offshore, and the presence of right whales, relative to all of the other fisheries and their associated gear.

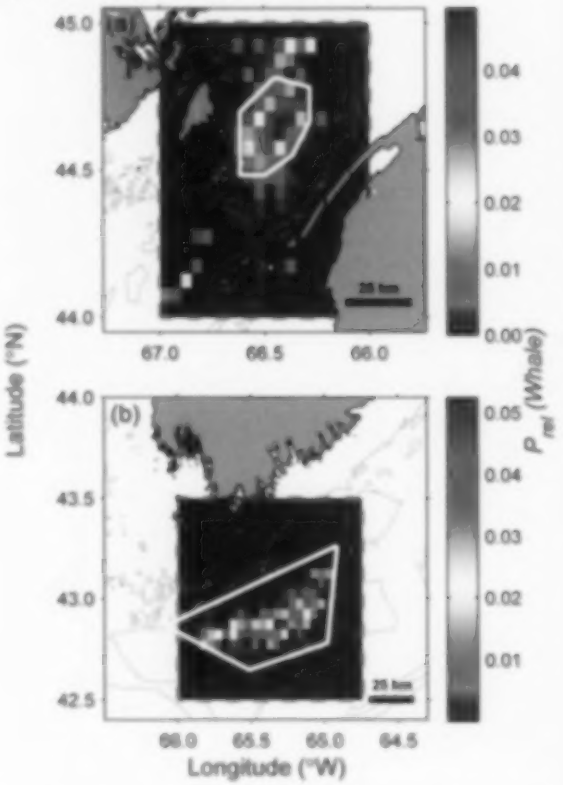
Although there is low temporal coincidence between the lobster fisheries and the right whales, mostly attributable to the lobster fishing seasons being out of phase with the presence of the whales, we have included both the offshore and inshore fisheries in the remaining analyses. The data limitations described above for the inshore-lobster fishery (lower spatial and temporal resolution and nonreporting of the number of sets fished) prevent direct comparisons between the inshore lobster fishery and any of the other fisheries we address.

Relative probability of observing a right whale

During summer in the Bay of Fundy study area, right whales are most concentrated in the Grand Manan Basin (Fig. 4*a*), and overall the chance of observing a right whale within the Critical Habitat is 68% (see also Vanderlaan et al. 2008). This corresponds to a 23-fold greater chance, on average, of observing a right whale inside the Critical Habitat than outside. There are slightly elevated relative probabilities north of the Critical Habitat that is bounded by the 100 m isobath and a relatively small probability of observing a right whale to the south, a region of ingress and egress of right whales to and from the Bay of Fundy.

In the Roseway Basin study area, the whales are most concentrated within the Critical Habitat (Fig. 4*b*) during summer, and there is an overall 99% chance of observing a right whale within the Critical Habitat, corresponding to a 73-fold greater chance of observing a right whale within the Habitat than elsewhere in the study area.

Fig. 4. Bathymetric (100, 200, 500, and 1000 m isobaths) charts illustrating the relative probability of observing a right whale, P_{rel}(Whale), within each of the (*a*) Bay of Fundy and (*b*) Roseway Basin study areas (pink dashed-line rectangles) and their associated right whale Critical Habitat (white solid-line polygons).



Gear threat and risk during the summer-resident period

Pelagic hook-and-line

Pelagic hook-and-line gear is deployed in a concentrated manner along the shelf break of the Scotian Shelf and Georges Bank (Supplemental Fig. S1*a*[1]). The relative threat from the amount of gear associated with this fishery is very small in the both the Fundy and Roseway Basin study areas, contributing 0.014% and 0.51%, respectively, to the overall Scotia–Fundy relative threat.

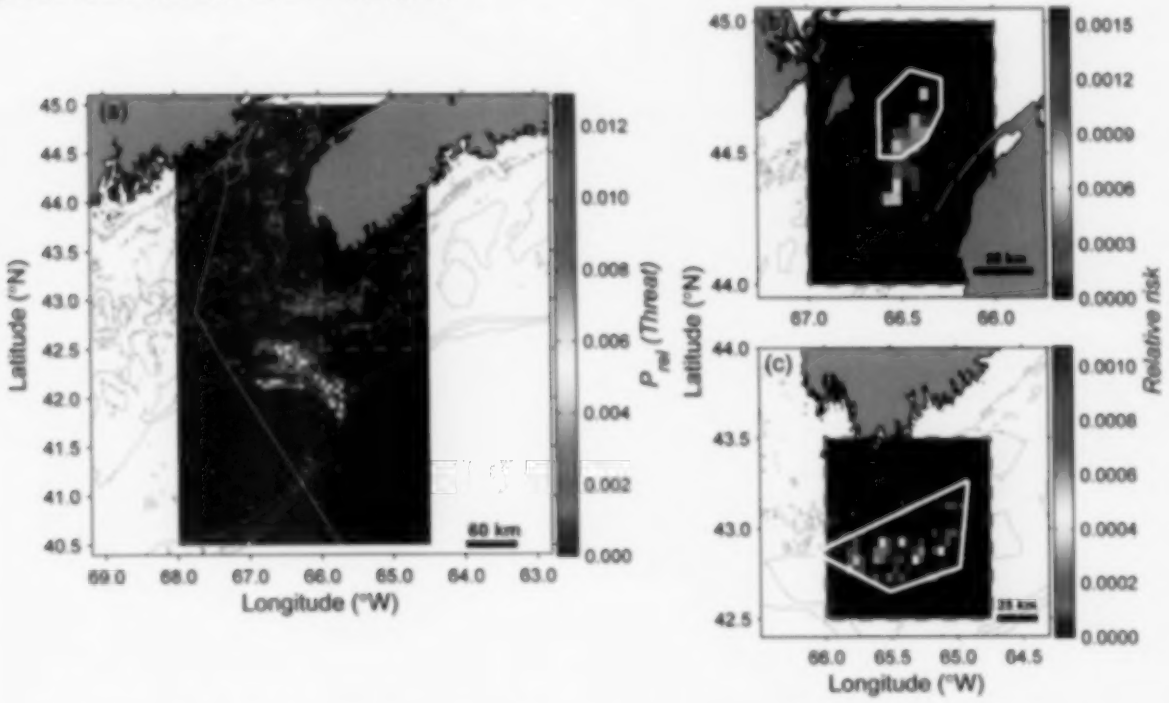
The fishery is not prosecuted in the Fundy area, and thus there is virtually no risk of lethal entanglement to right whales (Supplemental Fig. S1*b*[1]). In the Roseway area, there is one grid-cell (~23 km^2) that is associated with high relative risk from pelagic hook-and-line gear, and it is contained within the Critical Habitat (Supplemental Fig. S1*c*[1]).

Groundfish hook-and-line

Groundfish hook-and-line gear is deployed throughout the larger Scotia–Fundy study domain, and the greatest relative threat is located along the northeast slope of Georges Bank and in the Northeast Channel (Fig. 5*a*). There is elevated

[1]Supplementary data are available with the article through the journal Web site at http://www.nrcresearchpress.com/doi/suppl/10.1139/f2011-124.

Fig. 5. Bathymetric (100, 200, 500, and 1000 m isobaths) charts illustrating (*a*) the Scotia–Fundy study area, the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (white solid line), the Bay of Fundy and Roseway Basin study areas (pink dashed-line rectangles), and the relative threat of entanglement in groundfish hook-and-line gear over the July through October period and the relative risk of lethal entanglement in each of the (*b*) Bay of Fundy and (*c*) Roseway Basin study areas (pink dashed-line rectangles) and their associated right whale Critical Habitat (white solid-line polygons).



threat in the lower Bay of Fundy and in the southwest Scotian Shelf region including Browns Bank and Roseway Basin. For the entire Scotia–Fundy area, 4.4% of the relative threat is located in the Fundy study area and 17% in the Roseway study area.

The highest relative risk of lethal groundfish hook-and-line entanglement in the Fundy area occurs within the Grand Manan Basin (Fig. 5*b*), and the average relative risk inside the Critical Habitat is 17-fold greater than outside. The Critical Habitat in the Fundy area does not encompass the majority of the risk, as it extends well to the south. In the Roseway Basin study area, the Critical Habitat encompasses virtually all of the risk, where on average it is two orders of magnitude greater than outside the Habitat; i.e., risk of lethal entanglement inside the Critical Habitat is 90-fold greater than outside (Fig. 5*c*).

Hagfish traps

The hagfish-trap fishery is a very small fishery relative to the other fisheries we examined. The total number of sets for any given month over the 9-year study period is smaller, by one to three orders of magnitude, relative to the other fisheries. The majority of hagfish traps are deployed in June through September when they coincide with the presence of right whales (see Fig. 2). Sixty-three percent of the threat from this fishery is located within the Roseway study area, and an additional 11% is located in the Fundy area northwest of the Critical Habitat (Supplemental Fig. S2*a*[1]).

In the Fundy study area, 2% of the grid-cells (~173 km^2) have relative risk values greater than zero, and there is zero relative risk of lethal entanglement with hagfish-trap gear in the Critical Habitat (Supplemental Fig. S2*b*[1]). The few grid cells associated with risk are west and northwest of the Critical Habitat and are located where the probability of observing a right whale is low. In the Roseway study area, the risk is greatest in the northeast sector of the Critical Habitat (Supplemental Fig. S2*c*[1]). Although the average relative risk within the Critical Habitat is 23-fold higher than outside, it is derived from very few sets, amounting to 116 over 9 years during the summer-resident period.

Crab traps

The majority of the threat posed by crab-trap gear to right whales in the Scotia–Fundy study area during July through October is outside the Fundy and Roseway study areas. Most threat is located southwest of Grand Manan Island and along the margins of the Jordan and Crowell basins and along the shelf break of Georges Bank and the Scotian Shelf (Supplemental Fig. S3*a*[1]). The relatively large amount of the gear deployed just south of Grand Manan Island extends well into the "grey zone" (Cook 2005). However, owing to the very low relative probability of observing a right whale in this area, there is minimal risk of crab-gear entanglement (but see below). Overall, for the Scotia–Fundy study area, 9.5% of crab-gear threat is within the Fundy study area and 0.39% is within the Roseway study area.

The relative risk to right whales of lethal entanglement in crab-trap gear is partially contained within the Critical Habitat of the Fundy study area, where it is generally elevated just south of the Critical Habitat (Supplemental Fig. S3*b*[1]), a region of whale ingress and egress to and from the Grand Manan Basin. Because of the aggregated nature of the whales, the average relative risk inside the Critical Habitat is approximately fourfold greater than outside. Risk is entirely contained within the Roseway Critical Habitat (Supplemental Fig. S3*c*[1]), where it is limited to seven grid-cells (~158 km^2). There is virtually no risk to right whales of entanglement in crab-trap gear outside the Roseway Critical Habitat in the study area.

Groundfish gillnets

Groundfish-gillnet gear is deployed throughout the larger Scotia–Fundy study domain (Supplemental Fig. S4*a*[1]). Outside the Fundy and Roseway study areas, the majority of the relative threat is located east of Jordan Basin and north of Crowell Basin. There is some threat of entanglement within the two smaller study areas, with the Fundy and Roseway study areas contributing 12% and 8.2%, respectively, to the overall entanglement threat in the Scotia–Fundy study area.

The majority of the relative risk in the Fundy study area is contained within the Critical Habitat (Supplemental Fig. S4*b*[1]). On average, inside the Critical Habitat, the relative risk is 7.5-fold greater than outside. In the Roseway study area, few grid cells (~4% = 475 km^2) are associated with a measurable risk of lethal entanglement (Supplemental Fig. S4*c*[1]); the average relative risk inside the Critical Habitat is threefold greater than outside.

Offshore lobster traps

There are four major concentrations of offshore lobster fishing activities in the Scotia–Fundy study area: along the northeast margins of the "grey zone" southwest of Grand Manan Island, the northeast margins of Crowell Basin, the southeast margin of Georges Bank, and the southwest shelf break of the Scotia Shelf (Supplemental Fig. S5*a*[1]). Some fishers who report as part of the offshore fishery also deploy lobster gear in the Bay of Fundy and Roseway Basin study areas; i.e., the deployments are actually associated with the inshore fishery (below). For the entire Scotia–Fundy study area, 5.0% of the relative threat is located in the Fundy study area and 1.4% in the Roseway study area.

On average, the risk of lethal entanglement is three orders of magnitude greater in the Bay of Fundy Critical Habitat than outside, and this corresponds to offshore fishers reporting gear deployments in three grid cells in the Grand Manan Basin (Supplemental Fig. S5*b*[1]). These reported locations are most likely erroneous, as the offshore fishery is prohibited from fishing in this area, and the removal of these cells from the risk analyses reveals that there is no risk of a lethal entanglement in offshore lobster gear in the Bay of Fundy Critical Habitat. There is no measurable risk of entanglement from offshore lobster gear in the Roseway Basin study area (Supplemental Fig. S5*c*[1]).

Inshore lobster traps

During the resident period for right whales (July through October), the inshore lobster fishery reports gear deployment along the northwest coast of Nova Scotia in the Bay of Fundy, and then only in the last 2 weeks of October; consistent with licensing regulations (Fig. 6). There are no other areas in the Scotia–Fundy study area where the inshore lobster fleet reports the hauling of lobster traps during July through October.

When the above gear-threat estimates for the entire Scotia–Fundy study area are standardized and proportionally partitioned within gear type among the migration and resident periods, all but the inshore and offshore lobster gear present the greatest threat (≥70%) during the summer-resident period (Table 1). Conversely (and consistent with Figs. 2*c* and 3*c*), lobster gear presents the least amount of threat (≤26%) during the summer-resident period, and for inshore lobster the threat during spring (85%) is comparable to that of all the non-lobster fisheries during summer (Table 1). These latter estimates highlight the degree of threat presented by most gear types during the spring-immigration period relative to the autumn-emigration period.

Gear threat in the Scotia–Fundy study area during seasonal migrations

The standardized gear-threat estimates (eq. 6 above) and comparisons below are based on the relative threat presented by each gear type deployed in the Scotia–Fundy study area but only for the spring-immigration (May and June) and autumn-emigration (November and December) periods that bracket the summer-resident period (July though October).

Pelagic hook-and-line

Virtually all (96%) of the threat from the pelagic hook-and-line fishery is present during the spring-immigration period (Supplemental Fig. S6[1]), and the majority of the threat is concentrated in a rectangular area (40.5°N to 42.5°N and 64.5°W to 66°W) south of the shelf break on Georges Bank and the southwest Scotian Shelf (Supplemental Fig. S6[1]). Though rarely surveyed, <0.1% of all right whale sightings (survey and opportunistic over the period 1978 though 2008) occur in this area.

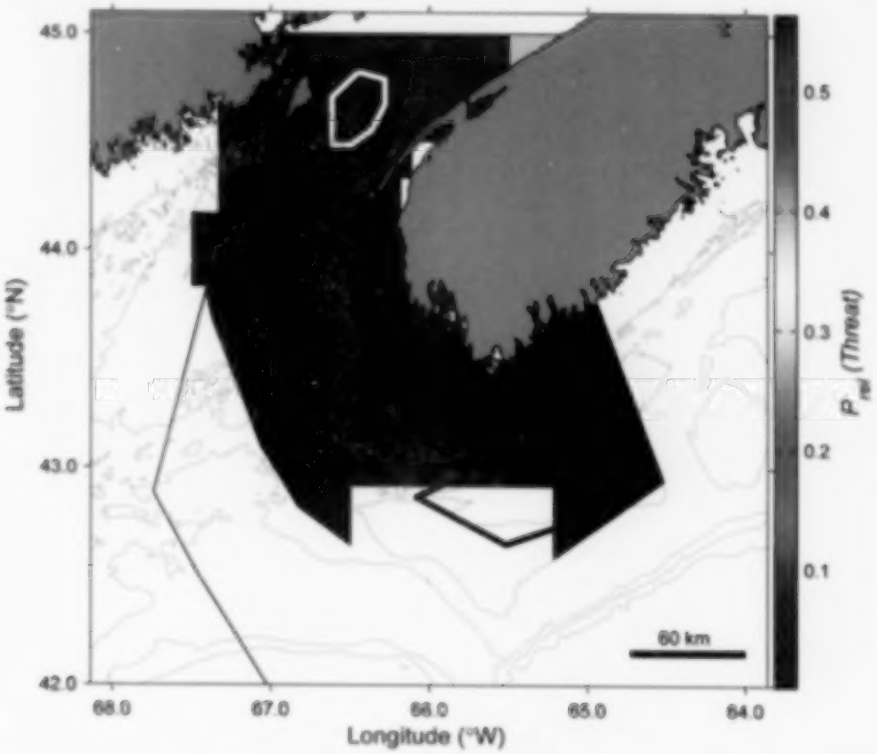
Groundfish hook-and-line

Although the amount of gear deployed by the groundfish hook-and-line fishery poses a broadly distributed threat during the summer (Fig. 5), it is even more broadly distributed during the spring-immigration period (Fig. 7*a*), when it accounts for 61% of the threat compared with 39% of the threat during the autumn-emigration period (Table 1). During the spring period, the majority of the threat is located in the Northeast Channel where it extends to the Roseway Basin and Browns Bank region and through to the lower Bay of Fundy and Grand Manan Basin. Gear threat during the autumn period is located primarily in the Northeast Channel, along the northeast margin of Georges Bank and in the Roseway Basin region (Fig. 7*b*). From a gear-set perspective, there are 2.5-fold more 3′ grid cells containing sets during the immigration period than during the emigration period.

Hagfish traps

The amount of hagfish-trap gear also poses the greatest threat (95%) during the spring period (Table 1; Supplemental Fig. S7*a*[1]) and the remaining 5% during the autumn period

Fig. 6. Bathymetric (100, 200, 500, and 1000 m isobaths) chart illustrating the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (grey solid line), right whale Critical Habitat (white and black solid-line polygons), and the relative threat of entanglement in inshore lobster gear (based on trap hauls) over the July through October period. Note the lower resolution and irregular grid structure (black solid line) relative to all other fisheries.



(Supplemental Fig. S7*b*[1]). The majority of the threat in both periods is located within the Roseway Basin study area.

Crab traps

For the migration periods, 79% of the threat associated with the amount of crab-trap gear in the Scotia–Fundy study area is present during the spring-immigration period (Table 1), although distributed well outside the smaller Fundy and Roseway study areas (Supplemental Fig. S8[1]). The threat is concentrated within and around Crowell Basin where it extends across possible whale-migration routes, as well as along the southwest margin of the Scotian Shelf and in spring along the southeast margin of Georges Bank.

Groundfish gillnets

As with the amount of crab-trap gear, groundfish gillnet gear-threat is greatest during the spring-immigration period (88%) relative to the autumn period (12%; Table 1), and though the threat is again distributed well outside the Fundy and Roseway study areas, it extends across possible whale-migration routes (Supplemental Fig. S9[1]).

Offshore lobster traps

Threat from the amount of offshore-lobster fishing gear during the spring-immigration (64%) and autumn-emigration periods (36%) is also located well outside of the Fundy and Roseway study areas (Table 1). The threat is most concentrated along the northeast margin of Georges Bank and the southwest margin of the Scotian Shelf in the spring and southeast of Crowell Basin in autumn where it extends across possible whale-migration routes (Supplemental Fig. S10[1]).

Table 1. Percent total relative threat to right whales of fishing-gear entanglement based on the amount of gear deployed in the Scotia–Fundy study region and partitioned within gear type among the spring-immigration (May through June), summer-resident (July through October), and autumn-emigration (November through December) periods (data in parentheses indicates partitioned between the spring and autumn periods only).

Gear type	Spring immigration	Summer resident	Autumn emigration
Pelagic hook-and-line	17 (96)	82	0.72 (4.1)
Groundfish hook-and-line	9.7 (61)	84	6.2 (39)
Hagfish trap	28 (95)	70	1.4 (4.8)
Crab trap	18 (79)	77	4.8 (21)
Groundfish gillnet	24 (88)	73	3.1 (11)
Offshore lobster trap	47 (64)	26	27 (36)
Inshore lobster trap	85 (89)	3.6	11 (11)

Inshore lobster traps

Because of the seasonal timing of the inshore-lobster fishery, threat from inshore lobster fishing gear (trap hauls) during the spring-immigration period is much greater (88%) than

Fig. 7. Bathymetric (100, 200, 500, and 1000 m isobaths) charts illustrating the Scotia–Fundy study area, the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (white solid line), the Bay of Fundy and Roseway Basin study areas (pink dashed-line rectangles), and the relative threat of entanglement in groundfish hook-and-line gear during periods of right whale (*a*) spring immigration (May through June) and (*b*) autumn emigration (November through December) to and from the study areas.

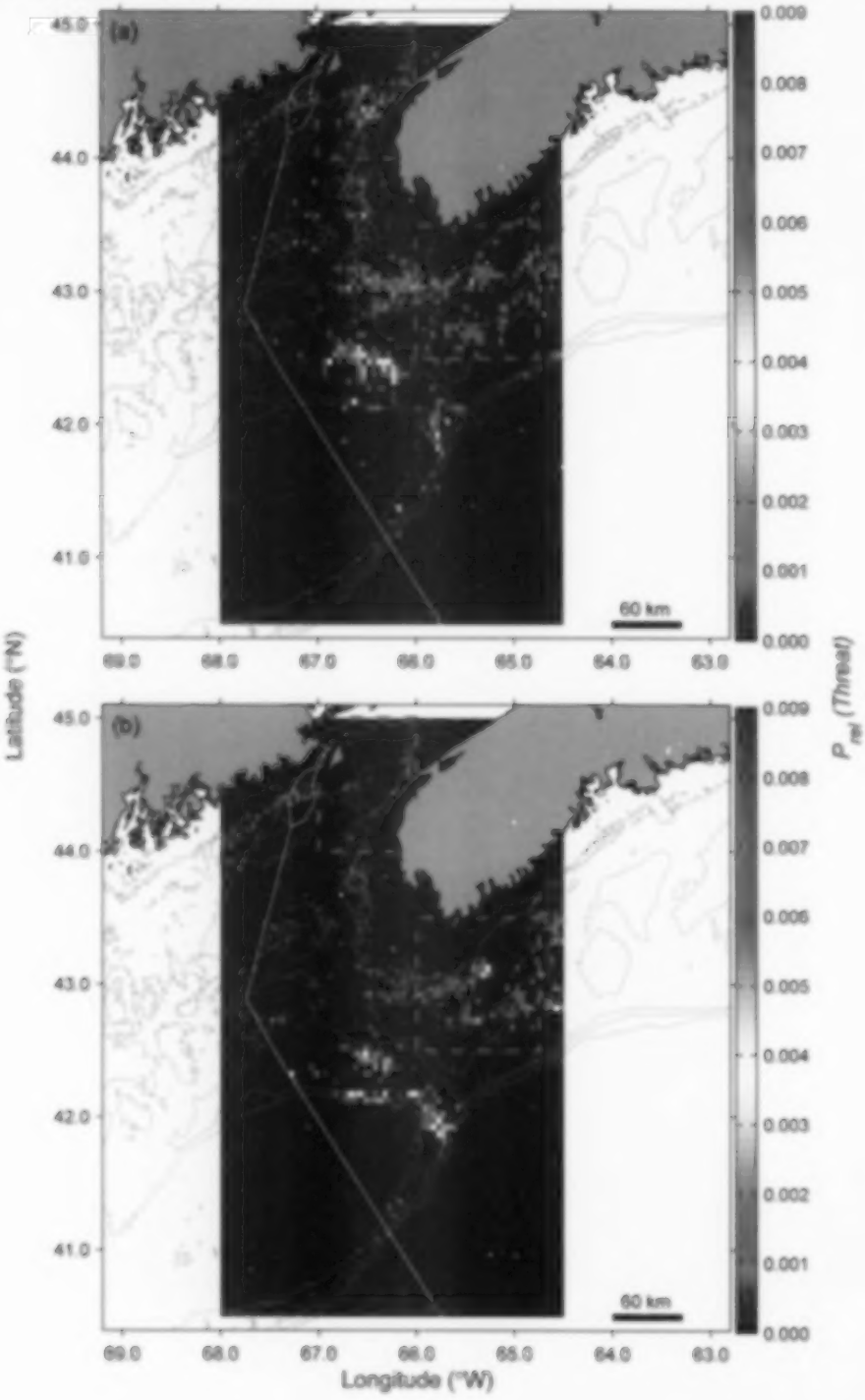
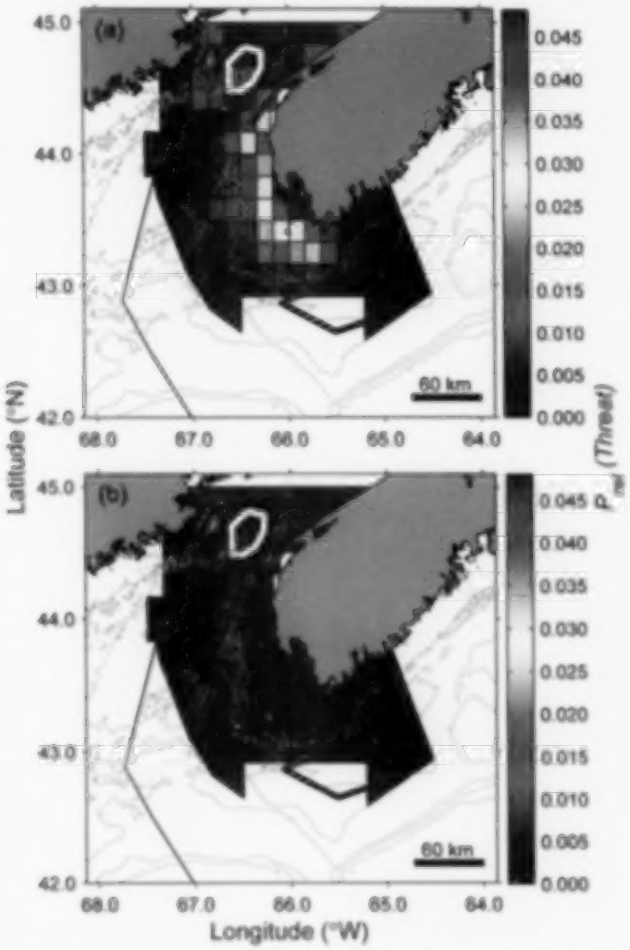


Fig. 8. Bathymetric (100, 200, 500, and 1000 m isobaths) charts illustrating the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (grey solid line), right whale Critical Habitat (white and black solid-line polygons), and the relative threat of entanglement in inshore lobster gear (based on trap hauls) during periods of right whale (*a*) spring immigration (May through June) and (*b*) autumn emigration (November through December) to and from the critical habitats. Note the lower resolution and irregular grid structure (black solid line) relative to all other fisheries.



during the autumn period (12%; Table 1) and is broadly distributed in the lower Bay of Fundy and extends southward to the margins of the Roseway Basin Critical Habitat (Fig. 8). The threat extends across potential routes for whales immigrating to the Fundy and Roseway critical habitats as well as whales moving between the two habitats. Gear threat during the autumn-emigration period is also broadly distributed, though it is ~8-fold lower than during the spring period.

In summary, for the gear threat assessed among the seven fisheries, and based on the amount of gear deployed and partitioned between the spring and autumn migration periods, the spring-immigration period is associated with the greatest overall threat in all cases (Table 1). The threat averaged over all gear types in spring is 82% ± 13% (median = 88%) relative to 18% ± 14% during the autumn. These estimates reflect the fact that during the autumn period, 23% of the grid-cells in Canadian waters of the Scotia–Fundy study area are associated with fishing-gear sets (excluding inshore lobster; no set data, low resolution), while during the spring-immigration period 56% of all grid-cells are associated with gear sets. These estimates are lower than that for the summer-resident period when 84% of all grid-cells are associated with fishing-gear sets. When we partition the percent contributions to the total threat among gear types based on gear sets at 3′ resolution (excluding inshore lobster gear) for each of the seasonal migration periods, it is offshore lobster-gear sets that contribute the most: 38% of the threat during the immigration period and 70% of the threat during the emigration period (Table 2). Secondarily, the groundfish hook-and-line sets represent 21% and 16% of the threat in spring and autumn, respectively.

Discussion

Gear threat and risk during the summer-resident period

The above results demonstrate that among the gear types analysed it is the amount of groundfish hook-and-line gear

Table 2. Percent total relative threat to right whales of fishing-gear entanglement based on the number of sets deployed in the Scotia–Fundy study region and partitioned among gear type sets within each of the spring-immigration (May through June), summer-resident (July through October), and autumn-emigration (November through December) periods and the percent of the total threat across all gear-type sets partitioned into each of the Bay of Fundy and Roseway Basin study areas.

Gear type	Spring immigration	Summer resident	Autumn emigration
Pelagic hook-and-line	5.7	9.7	0.36
Groundfish hook-and-line	21	42	16
Hagfish trap	0.95	0.69	0.080
Crab trap	16	19	9.6
Groundfish gillnet	17	18	3.5
Offshore lobster trap	38	9.6	70
Inshore lobster trap	—	—	—
Bay of Fundy	5.5	11	1.0
Roseway Basin	5.7	9.1	5.1

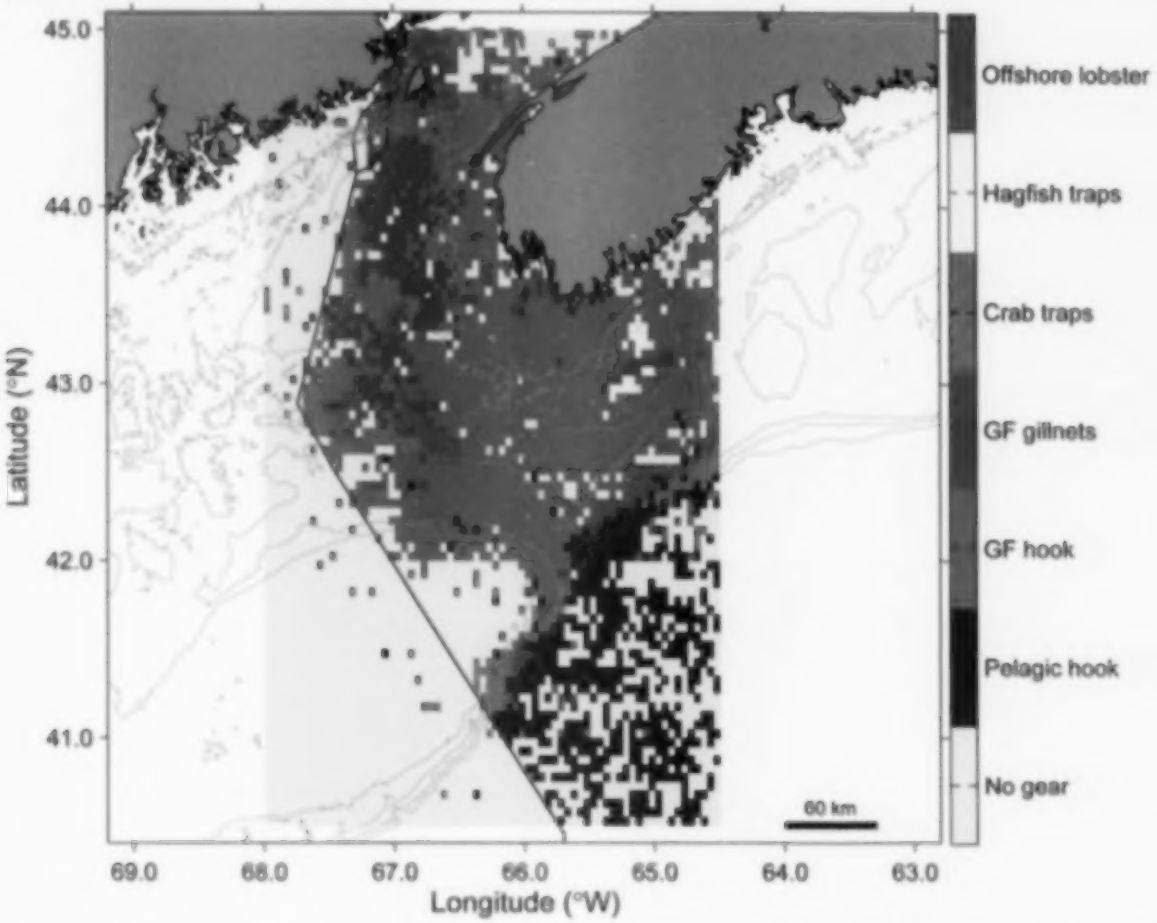
that poses the greatest overall relative threat (42%) to right whales during the July through October period in the Scotia–Fundy study area. This period is associated with the greatest probability of right whales being in the region. This fishery represents the majority of gear sets in the Scotia–Fundy study area, and the sets are widely distributed. This fishery has the second lowest SSW statistic that represents a high degree of temporal coincidence with right whales, consistent with the high and in-phase seasonal cross-correlation between right whales and gear deployment in the area. Compared with the other fisheries, the gear deployed for this fishery presents considerable and extensive risk of lethal entanglement in each of the Fundy and Roseway study areas where right whales are most aggregated during summer and autumn. The design and deployment of the gear provides the potential for entanglement, given the lengths of hook-laden line that typically measures 10^3 m or more for one or more strings that are anchored near bottom and are associated with buoyed end-lines that remain unattended in the water column for 24 h or more (Johnston et al. 2007). In many respects, the deployment configuration of the gear is similar to that of a lobster-trap trawl; i.e., comprising buoyed end-lines and an off-bottom profile.

While a number of gear modifications might reduce risk of lethal entanglement within the Bay of Fundy and Roseway Basin critical habitats (e.g., weak links, sinking groundlines), the simplest, most direct, and practical means of reducing groundfish hook-and-line risk to the whales may be to curtail sets within Critical Habitat over the July through October period (i.e., area-specific seasonal closure). The effects of such a closure are explored below as an illustration of the potential mitigation strategy. Area-specific closures, if implemented in the Roseway Basin and Bay of Fundy critical habitats, would result in the average annual displacement of 5.6% ± 1.0% and 0.30% ± 0.18%, respectively, of all sets deployed throughout the Scotia–Fundy study area. If the entire Bay of Fundy study area was considered for seasonal closure, it would remove virtually all risk of lethal entanglement in the gear during the summer-resident period. Such a closure would result in an average annual loss of 8.5% ± 3.5% of the total sets in Scotia–Fundy study area and for the most prevalent fish species a loss of 1.4% ± 1.1% of cod (*Gadus morhua*), 0.33% ± 0.18% of haddock (*Melanogrammus aeglefinus*), and 0.27% ± 0.44% of cusk (*Brosme brosme*) landings. If the above seasonal closure was implemented, catches for this fishery in the closed areas would represent small proportions of the Scotia–Fundy landings, and thus the economic impact may be marginal. The displacement of the small proportions of sets out of high-risk Critical Habitat would be to regions of considerably lower risk; i.e., the risk reduction in Critical Habitat would likely be far greater than the risk increase elsewhere.

Based on the number of sets, the crab-trap fishery represents 21% of the total number of sets and 19% of the relative threat in the Scotia–Fundy study area (Fig. 9). This fishery has been declining in the Scotia–Fundy study area, with the number of sets per year declining by an average of 60% over the 2005 through 2007 period. However, with the overall decline in the fishery, crab-trap deployments have increased in the Bay of Fundy, but not in the Critical Habitat. If a seasonal closure of an area slightly larger than the Critical Habitat (e.g., increase the boundaries of the Critical Habitat by 3′ to the south, west, and north) was considered for implementation, the majority of the risk would be removed. There have been seven crab-trap sets within the Fundy Critical Habitat over the 9-year study period, and relative to the 9825 sets in the Scotia–Fundy study area, a seasonal closure of this nature would have a minimal affect (0.07% of total sets) with an associated maximum affect on risk reduction. A similar seasonal closure of the Roseway Basin Critical Habitat would virtually eliminate the risk of crab-trap gear entanglement and would have a measurable but small impact on 0.17% of the total sets in the Scotia–Fundy study area during the summer-resident period. The impact on individual fishing licenses would likely have to be considered. As with groundfish hook-and-line gear, the risk reduction in Critical Habitat through displacement would be far greater than the risk increase elsewhere.

The groundfish-gillnet fishery appears to pose considerable gear-related threat (18% of total threat; Table 2) to right whales, as the majority of gear deployments in the Scotia–Fundy study area occur during the months of July though October, and sets are widely distributed throughout the study area, including the Bay of Fundy and Roseway Basin study areas (Fig. 9). To reduce the risk of lethal entanglement, a seasonal closure of the Fundy Critical Habitat could be considered because, on average, 0.35% ± 0.40% of annual Scotia–Fundy sets occur in the Habitat during the summer-resident period. Such a closure would reduce the risk that is, on average, 12.5-fold greater inside the Habitat than outside, with a presumedly small consequence to the fishery. A seasonal closure of the Roseway Critical Habitat would also reduce the risk of entanglement to near zero and would represent a loss of 3.9% of the total number of sets in the Scotia–Fundy study area during the July through October period. Such a closure would also represent an annual average loss of 1.9% ± 0.94% of pollock (*Pollachius virens*), 6.9% ± 4.8% of cod, and 1.2% ± 0.65% of white hake (*Urophycis tenuis*) landings in the Scotia–Fundy study area. Such a closure would likely have a marked effect on the groundfish gill-

Fig. 9. Bathymetric (100, 200, 500, and 1000 m isobaths) chart illustrating the Scotia–Fundy study area, the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (grey solid line), the Bay of Fundy and Roseway Basin study areas (pink dashed-line polygons), and the type of fishery with the greatest number of set deployments within each of the 3′ grid cells over the period July through October when pelagic (Pelagic hook) and groundfish (GF hook) hook-and-line, groundfish gillnet (GF gillnets), crab (Crab traps) and hagfish (Hagfish traps) traps, and offshore lobster (Offshore lobster) gear is set.



net fishery, though it would decrease the risk of lethal entanglement to near zero.

The pelagic hook-and-line fishery represents 9.7% of the total gear-threat in the Scotia–Fundy study area during the summer-resident period, and the majority of the gear is deployed along and beyond the Continental Shelf margin (Fig. 9). Less than 0.11% of sets (i.e., four sets over 9 years) for this fishery were deployed in the Bay of Fundy study area. Similarly, in the Roseway Basin study area 11 sets were deployed over the 9-year period. The three most prevalent species landed by the fishery were swordfish (*Xiphias gladius*) and bigeye (*Thunnus obesus*) and yellowfin (*Thunnus albacares*) tuna, and they are typically fished along and off the shelf break. A permanent closure of the pelagic hook-and-line fishery in each Critical Habitat would reduce risk to near zero with what appears to be negligible impact on the fishery.

The hagfish-trap fishery represents 0.1% of the total number of sets deployed in the Scotia–Fundy study area over the 9-year period (Fig. 9). The gear may represent one of the least threatening gear types, as there is only one trap and one buoy line for each set, and there were only 116 sets deployed over the study period. However, 63% of the threat from this fishery occurs within the Roseway study area, and the majority of the gear sets (61%) are deployed when the whales are aggregated in the area. A closure in the Critical Habitat would remove the risk of lethal entanglement but may have serious ramifications for the hagfish-trap fishery, as most of the effort is concentrated there. Relative to the other fisheries, seemingly few licenses would be affected, and displacement elsewhere would have near zero impact.

The offshore lobster fishery, during the summer-resident period, represents 9.6% of the total gear threat based on sets in the Scotian–Fundy study area. The majority of threat is distributed well outside the two critical habitats, as they are not enveloped by the offshore lobster management areas. Nevertheless, four sets in the Fundy and one set in the Roseway Critical Habitat areas were reported over the 9-year pe-

riod. We assume that these sets are erroneously reported. On this assumption, the offshore lobster fishery represents no threat to right whales inside Critical Habitat during the summer-resident period.

Based on the admittedly limiting nature of the inshore lobster logbook reporting data, our analyses show that there is not a measureable threat to right whales during the summer period in any part of the Scotia–Fundy study area, including Critical Habitat except within seven of the 10′ reporting-grids located east of 66°W in the Bay of Fundy. Over a 30-year period (1978 through 2008), and based on all right whale sighting data (survey and opportunistic; North Atlantic Right Whale Consortium 2008), there have been two right whales sighted east of 66°W in the Bay of Fundy. Barring unknown distributions of right whales in the Bay of Fundy, it is reasonable to conclude that the inshore lobster fishery constitutes no measureable threat to right whales during the summer period.

Gear threat during the spring- and autumn-migration periods

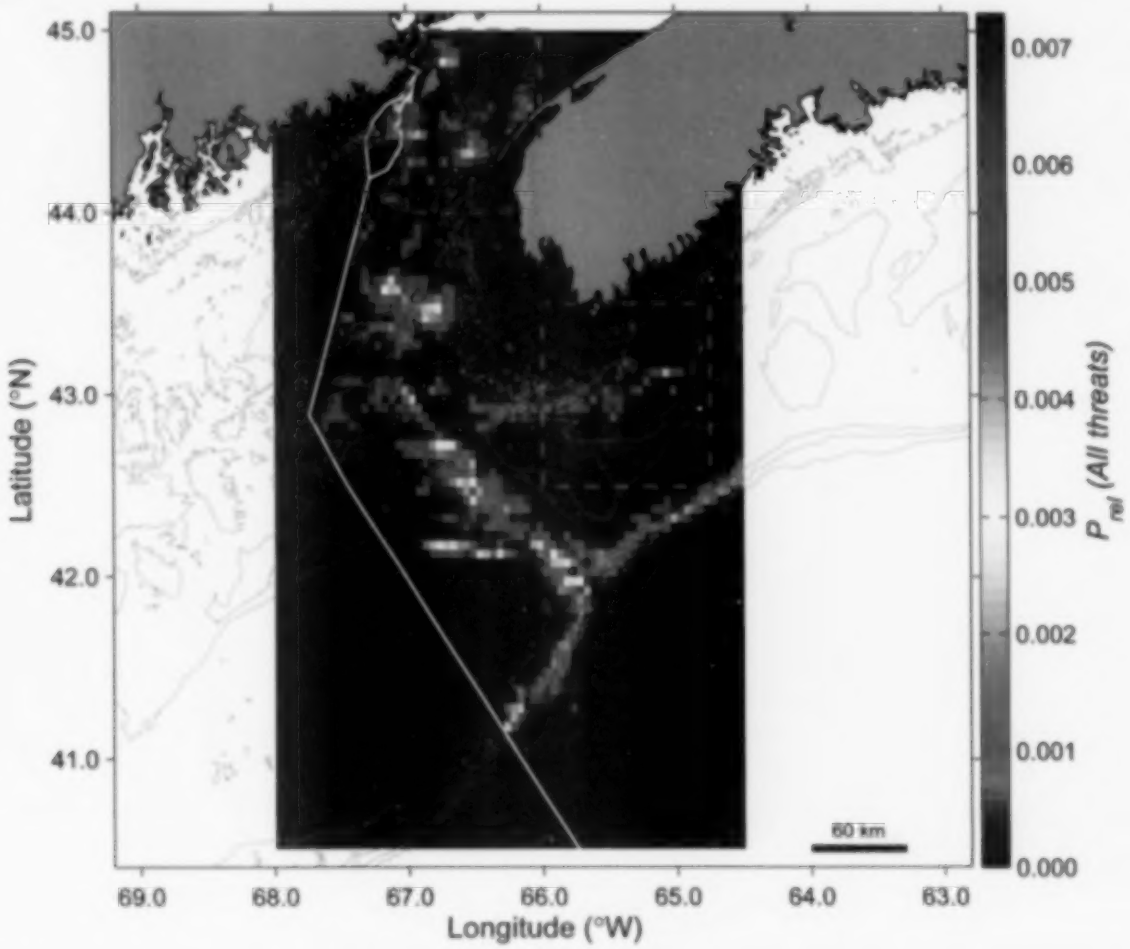
From a temporal perspective, and in light of the spatially related interpretations above that focus on the summer-resident period, it is clear that of the seven fisheries considered, all but the two lobster fisheries are strongly and positively cross-correlated and mostly in phase with the presence of right whales in the Scotia–Fundy study area. This led to suggestions that gear-specific seasonal or permanent closures for the other five fisheries in Critical Habitat could be considered to minimize measureable risk to the whales and with measurable consequences to each fishery. It is equally clear that the two lobster fisheries are strongly and negatively cross-correlated and out of phase with the presence of right whales during the summer-resident period when these two fisheries present negligible risk.

A comprehensive comparison of Figs. 2, 3, 7, 8 and Supplemental Figs. S6 through S10[1], along with Tables 1 and 2, leads to the conclusion that some of the most threatening gear to whales when migrating toward and away from the Fundy and Roseway regions, but not in Critical Habitat when whales are present, is that associated with inshore and offshore lobster traps. The threat is most prevalent during the spring-immigration period for each lobster fishery and less so but substantial during autumn emigration for the offshore fishery. In terms of total fishing-gear threat, the offshore lobster-trap fishery represents the greatest threat (70%) in the autumn period, when it is nearly double that relative to all gear types (except inshore lobster that is not quantifiable) in the spring. Further, four gear types (where measureable) are responsible for 92% of the threat during spring (38% offshore lobster, 21% groundfish hook-and-line, 17% groundfish gillnet, and 16% crab trap), but only two are responsible for 86% during the autumn period (70% offshore lobster and 16% groundfish hook-and-line). Outside the Fundy and Roseway study areas, we cannot estimate risk of lethal entanglement for any gear type, particularly during the immigration and emigration periods, as the right whale SPUE data are much too sparse in the Scotia–Fundy study area during these periods.

The above analyses indicate lobster gear poses a considerable threat to migrating right whales during the immigration and emigration periods, but less so for the summer-resident period. Therefore, a potential exists for substantial change in risk in the future if ongoing climate change induces spatial or temporal changes in right whale food production in and around Critical Habitat and (or) changes in the timing of immigration, emigration, and the length of the summer-residency period of right whales in Canadian waters. For example, a delay of just a few weeks in autumn emigration would likely result in a much larger coincidence between the whales and lobster gear associated with the autumn inshore lobster fishery that opens in early November and thus a greatly increased risk of lethal entanglement. Observations on the timing of migration for the eastern Pacific grey whale (*Eschrichtius robustus*) indicate such changes can occur. Rugh et al. (2001) used a data series over the period 1967 through 1999 to estimate a 1-week delay (6.8 ± 2.0 days, mean ± confidence interval) in the southbound migration of the whales subsequent to 1980. The change in timing was concurrent with the North Pacific "regime shift" of the late 1970s that is related to the influence of the Pacific Decadal Oscillation (Francis et al. 1998).

Although risk cannot be quantified when right whales are outside the Bay of Fundy or Roseway Basin study areas, or when migrating to, from, and among these and other regions, we can provide some insights into the spatial–temporal distribution of fishing-gear threat that the whales must navigate. When the whales are moving among the two feeding habitats and other regions during summer, and there is evidence of this from tagging data (Mate et al. 1997) and transition-matrix studies based on individually identified whales (Vanderlaan 2010), they are likely navigating and encountering widely distributed "fences" of fishing gear associated primarily with the groundfish hook-and-line and gillnet fisheries as well as the crab and offshore lobster fisheries (Fig. 9). When relative threat, based only on the number of sets, imposed by all fishing gear above (except inshore lobster) during summer is examined, the threat is extensive and generally parallels the isobaths (Fig. 10). Threat is greatest throughout the Northeast Channel and along the margins of the continental shelf, east of Jordan Basin, in Crowell Basin, and extending into the Bay of Fundy and Roseway Basin regions, each representing areas that right whales very likely transit when moving among summer-feeding habitats and other locations. During the summer-feeding period, the average annual number of sets in the Scotia–Fundy study area for all six fisheries is 5270 ± 480. Each set has at least one or two buoy lines, tens to hundreds of metres of float and (or) groundlines connecting tens to hundreds of traps and multiple net-panels, and hundreds to thousands of metres of hook-laden line. These gear deployments correspond to a daily average number of sets in July, August, September, and October of 52, 53, 40, and 26, respectively, in the Scotia–Fundy study area. On average, on any given day in August, a right whale swimming in the Scotia–Fundy study area could encounter 53 fishing-gear sets, each associated with multiple lines, and nets or traps. Approximately 84% of all grid-cells in Canadian waters in the Scotia–Fundy study area are associated with some type of gear over the 9-year study period (Fig. 9). There is a 3.7-fold increase in the number of grid-cells associated with fishing gear during the summer-feeding period (July through October) over that of the autumn-emigration

Fig. 10. Bathymetric (100, 200, 500, and 1000 m isobaths) chart illustrating the Scotia–Fundy study area, the Canadian Exclusive Economic Zone boundary and "grey zone" polygon (white line), the Bay of Fundy and Roseway Basin study areas (pink dashed-line polygons), and the relative threat of entanglement in any fishing gear, except inshore lobster, based on the number of sets deployed over the July through October period.



period (November, December) and a 1.5-fold increase over that of the spring-immigration period (May, June).

In comparing the migration periods of the right whale, there is almost a 2.5-fold increase in the number of grid-cells that are fished in the spring-immigration period relative to the autumn-emigration period, and this excludes the thousands of inshore lobster trap deployments. Accordingly, as the whales migrate from Cape Cod Bay and the Great South Channel (Winn et al. 1986) and move through the Scotia–Fundy study area during the spring-immigration period, they are about three times more likely to encounter fishing gear than in the autumn when they emigrate. Until better information is available on the probabilistic movements of the animals when they migrate through the Scotia–Fundy region, it is not possible to ascertain the nature of protective measures that would be most effective in reducing the risk of a fishing-gear entanglement outside the critical habitats during the migration periods. Efforts are underway to determine the time and space probabilistic distributions of right whales throughout the Gulf of Maine and Scotia–Fundy region over the entire year (S. Brillant, Canadian Wildlife Federation, 350 Michael Cowpland Drive, Kanata, ON K2M 2W1, Canada, unpublished data), and when completed, fishing gear risk analyses in Canadian waters in regions external to critical habitat will be afforded. Until completed, gear threat offers the only quantitative measure of potential risk.

Estimating the lethality of an entanglement

We estimated that a right whale has ~1% chance of a lethal injury resulting from a fishing-gear entanglement and that 97 ± 39 entanglements may occur annually (eq. 9 above). This estimate is consistent with Moore et al. (2006), who estimate that between 49 and 136 right whales are entangled annually based on 1986–2005 reports. The estimate can be calculated differently following Knowlton et al. (2008) by considering only the most recent period of 1993 through 2004, when ≥50 right whales were "adequately photographed" each year. Using this shorter time series, we

estimated an average annual entanglement rate of 0.25 ± 0.081 (again based on a population of 350 individuals) corresponding to annual estimate of 87 ± 29 entanglements sufficient to cause scarring, as opposed to 0.28 ± 0.11 and 97 ± 39, respectively. Therefore, a second estimate of the probability of a lethal entanglement (death occurred, was presumed, or was expected) using eq. 9 can be estimated as 23/1720 = 0.013 ± 0.0044: again, approximately a 1% chance of a lethal entanglement, consistent with our first estimate of lethality. Though these two estimates are marginally different for lethality in our risk estimations, we used 0.012 for three reasons. First, it is based on the longer data series (greater N and the resultant advantages offered by central tendency) that covers a greater period of variation in gear type, gear amount, and distribution pattern, as well as whale abundance and distribution pattern. Second, our analyses showed that the coefficient of variation, based on the annually incrementing mean and variance, stabilized at 0.4 in 1995 and remained virtually constant (stationary) thereafter; i.e., entanglement probability based on scarring reports has remained near constant at 0.28 over the data period. The third reason is that if we base the estimate on the more recent period (shorter series as in Knowlton et al. 2008) and incorporate an increase in whale population abundance to at least 400 (Pettis 2009), the estimate remains at 0.012.

Our P(Lethal|Encounter) estimate is lower than that estimated by Kraus (1990) at 4.3% annually, and it is assumed that such estimates are underestimates (Knowlton and Kraus 2001; Moore et al. 2004, 2006) given that for the entire species it is estimated that only 17% of all mortalities are known (Kraus et al. 2005). We can adjust the number of whales that have died due to entanglements by using a method similar to that of Vanderlaan et al. (2009) to account for the 17% mortality detection. If we remove the ψ term from eq. 4 in the above-cited paper (i.e., no dead whales where death may be attributed to entanglement; Moore et al. 2004), then we estimate the total number of lethal entanglements to be 66 over a 32-year period. This corresponds to P(Lethal|Encounter) = 0.021 using the above number of annual entanglements or 0.022 using the median number of annual entanglements from Moore et al. (2006). One other estimate is possible by using the qualitative statistic from Johnson et al. (2007), who state that nearly every year a right whale either dies from a fishing-gear entanglement or disappears when still entangled. This "statistic" and the median value of the estimated number of entanglements (Moore et al. 2006) approximate a P(Lethal|Encounter), assuming a "disappeared" whale is dead, of 0.011, equivalent to our initial estimate above. The latter estimate decreases slightly to 0.010 if we use the estimated number of entanglements (97 ± 39), though it remains within the limits of uncertainty. Finally, if we adjust, varyingly, for unknown, undetected, presumed, and expected deaths, we estimate there is a 1%–2% chance of an entangled right whale dying. Given all of the above, we can reasonably conclude that the probability that an entanglement proves lethal is between 1% and 2% and perhaps as high as 4% (Kraus 1990), and note from our analyses of the scarring reports above that it has remained relatively constant over at least a decade.

We have taken a precautionary approach to estimating risk of a lethal fishing-gear entanglement by assuming lethality is constant across all gear types, primarily because the data available among gear types is far too sparse to validate the assumption in a statistically robust manner. We argue that the assumption is reasonable, until statistically shown otherwise, for seven reasons. (1) All parts of fixed fishing-gear types have been recovered from right whales and humpback whales (Johnson et al. 2007). (2) Of the gear types we examined here, each has been associated with a known large-whale fishing-gear entanglement (Baird et al. 2002; Johnson et al. 2005; Neilson et al. 2009). (3) There is a statistically small number of right whale entanglements reported in the literature where gear type is identified (14/31 = 45%; Johnson et al. 2005). (4) There is a very large number of right whales that exhibit scarring events consistent with fishing-gear entanglements, and each results from one or more unknown gear type (Knowlton et al. 2008). (5) Of 30 necropsied right whales, death was attributed to entanglement in four cases, of which two were associated with a particular gear type (Moore et al. 2004). (6) There is a suite of problems associated with identifying fishing gear involved in a given entanglement, and there is no known means by which to standardize entanglement rate across fishing-gear effort (Johnson et al. 2005, 2007). (7) Although Johnson et al. (2005) provide an analysis of the gear associated with entangled right and humpback whales that could provide insights into risk across gear types, we concur with their conclusion that the threat posed by a specific gear type cannot be estimated without adjusting reported entanglements by fishing effort that is currently not available over the entire range of right whales.

Relative risk estimates

We emphasise that the relative risk estimates we provide, based on constant lethality across gear type (eq. 8), can be easily converted to a gear-specific lethality when such estimates are resolved. Accordingly, all spatial representations of relative risk can be reinterpreted simply by modifying the estimates within gear type by using the "new" gear-specific lethality. The encounter probabilities we provide and use to estimate risk need not be re-estimated. Until there is more information on the mechanics of a fishing-gear entanglement, the likelihood of each gear type entangling a right whale, the likelihood of self-disentanglement by gear type, and the standardization of entanglement rates by fishing effort across the right whale range, it is not possible to estimate in any robust manner the lethality of an entanglement among the various gear types. New research is underway using advanced modeling techniques to determine the mechanics of right whale entanglements among gear types (L.E. Howle, Belle-Quant Engineering, PLLC, 7813 Dairy Ridge Road, Mebane, NC 27302, USA, personal communication, 2010) that may offer insights regarding gear-specific lethality.

We have estimated the risk of lethal fishing-gear entanglements in defined right whale aggregation areas and the seasonal threat of fishing gear throughout the Scotia–Fundy study area. In each case, the threat estimates are underestimates for right whales throughout their known range, as we have examined only Canadian fishing-gear threat and risk in the Scotia–Fundy study area, and we have not included fishing gear deployed in USA waters and elsewhere in Canadian waters. West of the Exclusive Economic Zone boundary

(Hague Line), there is an unknown amount of threat and risk from USA fisheries that may be larger than we estimate from Canadian fisheries in the Scotia–Fundy study area. For example, the lobster fishery in Maine is active year-round and uses 8- to 9-fold more traps at any given time than those used in Lobster Fishing Area 34 (south and west of Nova Scotia and western Bay of Fundy; inshore lobster) in Canadian waters (Myers et al. 2007). Entanglement mitigation through gear modifications for the lobster fishery in the USA have been adopted (National Oceanic and Atmospheric Administration 2007*b*; Maine Department of Marine Resources 2008).

We suggested that fishery closures offer a simple and direct method of reducing the risk of gear entanglements in right whale summer-feeding habitats in Canadian waters. Various regulatory modifications to fishery operations (e.g., seasons and boundaries) and their associated gear (e.g., number of buoys, sinking and neutrally buoyant lines, and weak links) have been adopted in a number of fisheries in the USA, including gillnet and trap or pot fisheries, to protect whales in general and right whales in particular (National Oceanic and Atmospheric Administration 2007*a*, 2007*b*; Knowlton et al. 2008). Such regulations are considered in some cases to be operationally ineffective and inadequate for reducing risk to right whales (Levesque 2009*a*) and in other cases provide various conservation benefits (Levesque 2009*b*). Most gear modifications are based on the assumption that they will reduce the entangling nature of gear. As we know little of the mechanics of a fishing-gear entanglement — how it occurs or how a whale frees itself once entangled — it is difficult to predict how and which gear modifications are most likely to measurably reduce the risk of a lethal entanglement. The process of developing gear modifications is generally slow, is rarely scientifically tested for effectiveness (but see Brillant and Trippel 2010), is often frequented by failure (Johnson et al. 2007), and can take years to implement (Kraus et al. 2005; Levesque 2009*a*). Given the many unknowns concerning the effectiveness of gear modifications, area-specific seasonal closures may be the most effective means for reducing the risk of entanglements to right whales, at least in the short term.

Canada has yet to mandate any changes among the Scotia–Fundy fisheries to reduce the risk of entanglements to right whales. Gear modifications may reduce some portions of the gear in the water column and (or) make it easier for an entangled whale to shed the gear, though the effectiveness of gear modifications has yet to be demonstrated (but see Brillant and Trippel 2010). As a result, we have no information on the functional form of lethality in relation to gear modification within or among gear types. Unless gear lethality can be reduced to near zero, fishery closures may represent the most effective means of reducing the risk, as they remove the entirety of the risk in the closed area. We have suggested where fishery- and area-specific seasonal closure might prove most effective and suggest that in many cases they may have limited effect on the fishery in terms of the most prevalent landings. Seasonal closures may also prove beneficial by providing an opportunity for the biomass of the targeted standing stock to build (e.g., lobsters; Myers et al. 2007).

In many respects, when it comes to options for reducing human-induced right whale mortality, gear modifications vs. fishery closures are analogous to vessel-speed limits vs. vessel rerouting. In each case, the first option is designed to minimize the lethality of the event (entanglement or vessel strike), though it can still occur, whereas the second option is designed to minimize the probability of the event occurring, in which case lethality becomes secondary. Given that vessel rerouting has proven to be highly successful in being adopted by the shipping industry (Vanderlaan et al. 2008; Vanderlaan and Taggart 2009), it is possible that fishery closures, as opposed to gear modifications, will be adopted by the fishing industry, though we have no evidence. Canada should not delay in implementing changes to current fishing practices if reducing the risk of right whale entanglement is considered a desirable goal. Doing so may also help avoid the same predicament facing Mexico, where fishing "buy-outs" are being used (Morell 2008) in an attempt to reduced the gillnet fishery and preserve the critically endangered vaquita (*Phocoena sinus*).

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References

Baird, R.W., Stacey, P.J., Duffus, D.A., and Langelier, K.M. 2002. An evaluation of gray whale (*Eschrishtius robustus*) mortality incidental to fishing operations in British Columbia, Canada. J. Cetacean Res. Manage. **4**(3): 289–296.

Brillant, S.W., and Trippel, E.A. 2010. Elevations of lobster fishery groundlines in relation to their potential to entangle endangered North Atlantic right whales in the Bay of Fundy, Canada. ICES J. Mar. Sci. **67**(2): 355–364. doi:10.1093/icesjms/fsp231.

Brown, M.W., Allen, J.M., and Kraus, S.D. 1995. The designation of seasonal right whale conservation areas in the waters of Atlantic Canada. *In* Marine protected areas and sustainable fisheries. *Edited by* N.L. Shackell and J.H.M. Willison. Science and Management of Marine Protected Areas Association, Wolfville, N.S. pp. 90–98.

Brown, M.W., Kraus, S.D., Slay, C.K., and Garrison, L.P. 2007. Survey for discovery, science, and management. *In* The urban whale: North Atlantic right whales at the crossroads. *Edited by* S.D. Kraus and R. Rolland. Harvard University Press, Cambridge, Mass. pp. 105–137.

Brown, M.W., Fenton, D., Smedbol, R.K., and Merriman, C. Robichaud-Leblanc, K., and Conway, J.D. 2009. Recovery strategy for the North Atlantic right whale (*Eubalaena glacialis*) in Atlantic Canadian waters [online]. Species at Risk Act Recovery Series. Fisheries and Oceans Canada, Ottawa, Ont. Available from http://www.sararegistry.gc.ca/virtual_sara/files/plans/rs_north_atl_right_whale_0609_e.pdf.

Clapham, P.J., Young, S.B., and Brownell, R.L. 1999. Baleen whales: conservation issues and the status of the most endangered

populations. Mammal Rev. **29**(1): 37–62. doi:10.1046/j.1365-2907.1999.00035.x.

Cook, B. 2005. Lobster boat diplomacy: The Canada–US grey zone. Mar. Policy, **29**(5): 385–390. doi:10.1016/j.marpol.2004.05.010.

Francis, R.C., Hare, S.R., Hollowed, A.B., and Wooster, W.S. 1998. Effects of interdecadal climate variability on the oceanic ecosystems of the NE Pacific. Fish. Oceanogr. **7**(1): 1–21. doi:10.1046/j.1365-2419.1998.00052.x.

Fujiwara, M., and Caswell, H. 2001. Demography of the endangered North Atlantic right whale. Nature, **414**(6863): 537–541. doi:10.1038/35107054. PMID:11734852.

Gaskin, D.E. 1991. An update on the status of the right whale, *Eubalaena glacialis*. Can. Field Nat. **105**: 198–205.

Johnson, A., Salvador, G., Kenney, J., Robbins, J., Kraus, S., Landry, S., and Clapham, P. 2005. Fishing gear involved in entanglements of right and humpback whales. Mar. Mamm. Sci. **21**(4): 635–645. doi:10.1111/j.1748-7692.2005.tb01256.x.

Johnson, A.J., Kraus, S.D., Kenney, J.F., and Mayo, C.A. 2007. The entangled lives of right whales and fisherman: Can they coexist? *In* The urban whale: North Atlantic right whales at the crossroads. *Edited by* S.D. Kraus and R. Rolland. Harvard University Press, Cambridge, Mass. pp. 380–408.

Johnston, T.L., Smedbol, R.K., Serdynska, A., Vanderlaan, A., Hekel, N., Harris, L., and Taggart, C.T. 2007. Patterns of fishing gear in areas of the Bay of Fundy and southwest Scotian Shelf frequented by North Atlantic right whales. Can. Tech. Rep. Fish. Aquat. Sci. 2745.

Jönsson, P., and Wohlin, C. 2004. An evaluation of *k*-nearest neighbour imputation using likert data. Proceedings of the 10th IEEE International Software Metrics Symposium, IEEE Computer Society, Los Alamitos, Calif., USA. pp. 108–118.

Kaplan, S., and Garrick, B.J. 1981. On the quantitative definition of risk. Risk Anal. **1**(1): 11–27. doi:10.1111/j.1539-6924.1981.tb01350.x. PMID:11798118.

Knowlton, A.R., and Kraus, S.D. 2001. Mortality and serious injury of northern right whales (*Eubalaena glacialis*) in the western North Atlantic Ocean. J. Cetacean Res. Manag. **2**: 193–208. [Special Issue.]

Knowlton, A.R., Cooper, H.A., Hamilton, P.K., Marx, M.K., Pettis, M., and Kraus, S.D. 2008. Analysis of scarring on North Atlantic right whales (*Eubalaena glacialis*): monitoring rates of entanglement interaction: 1980–2004. Final report to the Northeast Fisheries Science Center, NMFS, Contract No. EA133F-03-SE-0323. New England Aquarium, Boston, Mass.

Kraus, S.D. 1990. Rates and potential causes of mortality in North Atlantic right whales (*Eubalaena glacialis*). Mar. Mamm. Sci. **6**(4): 278–291. doi:10.1111/j.1748-7692.1990.tb00358.x.

Kraus, S.D., and Rolland, R.M. (*Editors*). 2007. Right whales in the urban ocean. *In* The urban whale: North Atlantic right whales at the crossroads. Harvard University Press, Cambridge, Mass. pp. 1–38.

Kraus, S.D., Brown, M.W., Caswell, H., Clark, C.W., Fujiwara, M., Hamilton, P.K., Kenney, R.D., Knowlton, A.R., Landry, S., Mayo, C.A., McLellan, W.A., Moore, M.J., Nowacek, D.P., Pabst, D.A., Read, A.J., and Rolland, R.M. 2005. North Atlantic right whales in crisis. Science, **309**(5734): 561–562. doi:10.1126/science.1111200. PMID:16040692.

Levesque, J.C. 2009*a*. Establishment and closure of the directed Florida whiting (*Menticirrhus americanus*) fishery and implications for North Atlantic right whale (*Eubalaena glacialis*) conservation and management. Mar. Policy, **33**(2): 233–247. doi:10.1016/j.marpol.2008.07.003.

Levesque, J.C. 2009*b*. Characterization of the southeastern US black seabass (*Centropristis striata*) pot commercial fishery and implications for western North Atlantic right whale (*Eubalaena glacialis*) management and policy. Mar. Policy, **33**(1): 40–48. doi:10.1016/j.marpol.2008.04.007.

Lien, J. 1994. Entrapments of large cetaceans in passive inshore fishing gear in Newfoundland and Labrador (1979–1990). Rep. Int. Whaling Comm. **15**(Special issue): 149–157.

Mate, B.R., Nieukirk, S.L., and Kraus, S.D. 1997. Satellite-monitored movements of the northern right whale. J. Wildl. Manage. **61**(4): 1393–1405. doi:10.2307/3802143.

Maine Department of Marine Resources. 2008. Chapter 75 — Protected Resources, 22 July 2008, amended: 16 February 2009 – Section 02(A), October 26, 2010 – Section 03(A)(1)(*d*). Department of Marine Resources, Augusta, Maine, USA.

Moore, M.J., Knowlton, A.R., Kraus, S.D., McLellan, W.A., and Bonde, R.K. 2004. Morphometry, gross morphology and available histopathology in North Atlantic right whale (*Eubalaena glacialis*) mortalities (1970–2002). J. Cetacean Res. Manage. **6**(3): 199–214.

Moore, M.J., Bogomolni, A., Bowman, R., Hamilton, P.K., Harry, C.T., Knowlton, A.R., Landry, S., Rotstein, D.S., and Touhey, K. 2006. Fatally entangled right whales can die extremely slowly. Oceans'06 MTS/IEEE, Boston, Mass., 18–21 September 2006. ISBN: 1-4244-0115-1. IEEE, Piscataway, N.J., USA.

Morell, V. 2008. Conservation biology: Can the vaquita be saved? Science, **321**(5890): 767. doi:10.1126/science.321.5890.767. PMID:18687936.

Murison, L.D., and Gaskin, D.E. 1989. The distribution of right whales and zooplankton in the Bay of Fundy, Canada. Can. J. Zool. **67**(6): 1411–1420. doi:10.1139/z89-200.

Myers, R.A., Boudreau, S.A., Kenney, R.D., Moore, M.J., Rosenberg, A.A., Sherrill-Mix, S.A., and Worm, B. 2007. Saving endangered whales at no cost. Curr. Biol. **17**(1): R10–R11. doi:10.1016/j.cub.2006.11.045. PMID:17208167.

North Atlantic Right Whale Consortium. 2008. North Atlantic Right Whale Consortium sightings database, 22 July 2008. New England Aquarium, Boston, Mass.

Neilson, J.L., Straley, J.M., Gabriele, C.M., and Hills, S. 2009. Non-lethal entanglement of humpback whales (*Megaptera novaeangliae*) in fishing gear in northern Southeast Alaska. J. Biogeogr. **36**(3): 452–464. doi:10.1111/j.1365-2699.2007.01820.x.

National Oceanic and Atmospheric Administration. 2007*a*. Taking of marine mammals incidental to commercial fishing operations; Atlantic Large Whale Take Reduction Plan. 25 June 2007. Federal Register (USA), **72**(121): 34632–34642.

National Oceanic and Atmospheric Administration. 2007*b*. Taking of marine mammals incidental to commercial fishing operations; Atlantic Large Whale Take Reduction Plan Regulations; Final Rule. 5 October 2007. Federal Register (USA), **72**(193): 57104–57194.

Nowacek, D.P., Johnson, M.P., Tyack, P.L., Shorter, K.A., McLellan, W.A., and Pabst, D.A. 2001. Buoyant balaenids: the ups and downs of buoyancy in right whales. Proc. Biol. Sci. **268**(1478): 1811–1816. doi:10.1098/rspb.2001.1730. PMID:11522200.

Nowacek, D.P., Johnson, M.P., and Tyack, P.L. 2004. North Atlantic right whales (*Eubalaena glacialis*) ignore ships but respond to alerting stimuli. Proc. Biol. Sci. **271**(1536): 227–231. doi:10.1098/rspb.2003.2570. PMID:15058431.

Pettis, H. 2009. North Atlantic Right Whale Consortium annual report card (1 November 2007 – 30 April 2009). International Whaling Commission Scientific Committee Meeting, 22–25 June, Madeira, Portugal, 2009. SC/61/BRG11, International Whaling Commission, Cambridge, England.

Reeves, R.R., Smith, B.D., Crespo, E.A., and Notarbartolo di Sciara, G. (*Compilers*). 2003. Dolphins, whales and porpoises: 2002–2010 conservation action plan for the world's cetaceans. IUCN/

SSC Cetacean Specialist Group, IUCN, Gland, Switzerland, and Cambridge, UK.

Rosenbaum, H.C., Brownell, R.L., Jr, Brown, M.W., Schaeff, C., Portway, V., White, B.N., Malik, S., Pastene, L.A., Patenaude, N.J., Baker, C.S., Goto, M., Best, P.B., Clapham, P.J., Hamilton, P., Moore, M., Payne, R., Rowntree, V., Tynan, C.T., Bannister, J.L., and DeSalle, R. 2000. World-wide genetic differentiation of eubalaena: questioning the number of right whale species. Mol. Ecol. **9**(11): 1793–1802. doi:10.1046/j.1365-294x.2000.01066.x. PMID:11091315.

Rugh, D.J., Shelden, K.E.W., and Schulman-Janiger, A. 2001. Timing of the gray whale southbound migration. J. Cetacean Res. Manage. **3**: 31–39.

Species at Risk Act. 2002. Canada. Parliament. House of Commons. S.C. 2002, c. 29. An Act respecting the protection of wildlife species at risk in Canada. Last amended 27 October 2011. Public Works and Government Services Canada, Ottawa, Ont.

Vanderlaan, A.S.M. 2010. Estimating risk to the North Atlantic right whale (*Eubalaena glacialis*) from ocean-going vessels and fishing gear. Ph.D. thesis, Department of Oceanography, Dalhousie University, Halifax, N.S., Canada.

Vanderlaan, A.S.M., and Taggart, C.T. 2009. Efficacy of a voluntary area to be avoided to reduce risk of lethal vessel strikes to endangered whales. Conserv. Biol. **23**(6): 1467–1474. doi:10.1111/j.1523-1739.2009.01329.x. PMID:19775275.

Vanderlaan, A.S.M., Taggart, C.T., Serdynska, A.R., Kenney, R.D., and Brown, M.W. 2008. Reducing the risk of lethal encounters: vessels and right whales in the Bay of Fundy and on the Scotian Shelf. Endanger. Species Res. **4**: 283–297. doi:10.3354/esr00083.

Vanderlaan, A.S.M., Corbett, J.J., Green, S.L., Callahan, J.A., Wang, C., Kenney, R.D., Taggart, C.T., and Firestone, J. 2009. Probability and mitigation of vessel encounters with North Atlantic right whales. Endanger. Species Res. **6**: 273–285. doi:10.3354/esr00176.

Wiley, D.N., Moller, J.C., and Zillinskas, K.A. 2003. The distribution and density of commercial fisheries and baleen whales within the Stellwagen Bank national marine sanctuary: July 2001 – June 2002. Mar. Technol. Soc. J. **37**(1): 35–53. doi:10.4031/002533203787537384.

Winn, H., Price, C., and Sorensen, P. 1986. The distributional biology of the right whale (*Eubalaena glacialis*) in the western North Atlantic. Rep. Int. Whaling Comm. **12**(Special issue): 129–138.

Winn, H.E., Goodyear, J.D., Kenney, R.D., and Petricig, R.O. 1995. Dive patterns of tagged right whales in the great south channel. Cont. Shelf Res. **15**(4–5): 593–611. doi:10.1016/0278-4343(94)00061-Q.

Identification of putative migratory pheromones from Pacific lamprey (*Lampetra tridentata*)

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Abstract: Pacific lamprey, *Lampetra tridentata*, has declined precipitously throughout their range in the Columbia River basin of North America. Tribal Nations and Federal and State agencies are engaged in efforts to restore these fish. Understanding whether Pacific lamprey emit and detect migratory pheromones is particularly important for these restoration efforts. Using behavioural assays, we demonstrated that migratory adult Pacific lamprey are attracted to odors emanating from their larval conspecifics. We then identified putative pheromones released by larval Pacific lamprey. Chemical analysis of the conditioned water from larval lamprey using liquid chromatography – mass spectrometry (LC–MS) revealed that the Pacific lamprey can release petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), and petromyzonol sulfate (PZS). Electro-olfactogram studies further demonstrated that adult Pacific lamprey can smell those bile acid compounds. Our data strongly indicate that the Pacific lamprey employ a chemical communication system mediated by a mixture of bile acids, as evidenced by pheromonal functions of the bile acid compounds in guiding migratory adult sea lamprey to the spawning streams. Comprehensive understanding of the chemical communications involved in lamprey migratory behavior may lead to improved scientific approaches for restoration efforts.

Résumé : La lamproie du Pacifique, *Lampetra tridentata*, a diminué de façon rapide dans l'ensemble de leur aire de répartition dans le bassin du Columbia en Amérique du Nord. Les agences des nations tribales, des états et du gouvernement fédéral se sont engagées dans des efforts pour la restauration de ces poissons. Dans ces efforts de restauration, il est particulièrement important de connaître si les lamproies du Pacifique émettent et détectent des phéromones de migration. À l'aide de tests comportementaux, nous avons démontré que les lamproies du Pacifique migratrices sont attirées par les odeurs émises par les larves de leur propre espèce. Nous avons ensuite identifié les hormones putatives émises par les larves des lamproies du Pacifique. L'analyse chimique par chromatographie en phase liquide et spectrométrie de masse (LC–MS) de l'eau conditionnée par les larves de lamproies montre que les lamproies du Pacifique peuvent émettre du bisulfate de pétromyzonamine (PADS), du bisulfate de pétromyzostérol (PSDS) et du sulfate de pétromyzonol (PZS). Des l'électro-olfactogrammes démontrent en plus que les lamproies du Pacifique adultes peuvent sentir ces composés d'acides biliaires. Nos données indiquent clairement que la lamproie du Pacifique utilise un système de communication chimique par l'intermédiaire d'un mélange d'acides biliaires, comme le démontre la fonction de phéromone exercée par le mélange d'acides biliaires pour guider les lamproies marines migratrices vers leurs cours d'eau de fraie. Une compréhension globale des communications chimiques impliquées dans le comportement migrateur des lamproies peut mener à des approches scientifiques améliorées dans les efforts de restauration.

[Traduit par la Rédaction]

Introduction

The Pacific lamprey *Lampetra* (*Entosphenus*) *tridentata* has declined throughout much of its range in the Columbia River basin of North America (Close et al. 2002). Concerns about the declining numbers of Pacific lamprey led the state of Oregon, USA, to list them as a sensitive species in 1993, followed by protected status in 1996 (Kostow 2002) and reclassification as a species at risk in 2005 (ODFW 2006). In 2003, eleven conservation groups petitioned the US Fish and Wildlife Service (USFWS) to protect Pacific lamprey and three other species of lampreys under the Federal Endangered

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Species Act, but the listing was denied because of lack of sufficient information.

Pacific lamprey are a critical cultural food for indigenous peoples in the Columbia River Basin, and loss of harvesting opportunities (aboriginal rights) and culture associated with declining numbers of Pacific lamprey drew urgent calls to restore this fish in the Columbia River Basin. Acting to restore Pacific lamprey and improve knowledge about its status and biology, the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) began a Pacific lamprey reintroduction study in 1998 in the Umatilla River, Oregon, USA, where Pacific lamprey had been extirpated. Reintroduction of adults was initiated in 2000 and has continued to present. CTUIR research has demonstrated success of the first stages of reintroduction; however, persistence of the re-established population continues to be evaluated (Close et al. 2009). A better understanding of the migratory and reproductive biology of these fish is needed to determine factors affecting successful adult migration and re-establishment of a persistent population.

Chemical cues may be especially important in establishing a persistent population. One aspect of Pacific lamprey biology that is not understood is whether they produce and use migratory pheromones similar to sea lamprey, *Petromyzon marinus*. Migratory pheromones are the chemical cues used to guide the migration of some fish species such as lampreys. Previous studies suggest that migratory pheromones released by larval sea lamprey guide migratory adults into rivers. Sea lamprey larvae produce and release two bile acids, petromyzonol sulfate (PZS) and allocholic acid (ACA) that are detected by migratory adults (Li et al. 1995). Behavioral evidence indicated that these two bile acids function as migratory pheromone in conjunction with other odors (Bjerselius et al. 2000; Vrieze and Sorensen 2001). A recent study showed that two other sulfated bile acids, petromyzonamine disulfate (PADS) and petromyzosterol disulfate (PSDS), are components of the migratory pheromone in the sea lamprey (Sorensen et al. 2005). Fine and Sorensen (2008) confirmed that PZS, PADS, and PSDS are the main components of the sea lamprey migratory pheromone; however, ACA did not seem to play a role.

The Pacific lamprey may rely on chemical communication to find suitable spawning habitat and mates. Yun et al. (2003) provided evidence that larval Pacific lamprey can produce and release PZS and ACA. Fine et al. (2004) reported that PZS and ACA were detected by one adult Pacific lamprey using electro-olfactogram (EOG) recordings. Finally, Robinson et al. (2009) found that Pacific lamprey olfactory sensitivity to putative pheromones, PZS and 3-keto petromyzonol sulfate (3kPZS), occurred throughout their prolonged spawning migration, with highest sensitivity during early spawning migration. Although these studies helped our understanding of Pacific lamprey olfactory sensitivity to some of the putative sea lamprey pheromones, a complete suite of pheromones produced by Pacific lamprey has not been fully identified and characterized. In this paper, we describe the results from a series of experiments examining whether adult Pacific lamprey behavior is mediated by chemical cues from larval conspecifics and whether the Pacific lamprey olfactory system responds to putative bile acid chemical cues that were released by the larval Pacific lamprey.

Materials and methods

Behavioral responses to larval odors

Animals and study site

Pacific lamprey are a species of conservation concern, and limited numbers of animals were available for experimentation. Adult specimens (n = 6, 400–580 mm length) were collected on 30 June and 1 July 2008 from Salt and West Twin creeks (10–14 °C) during their upstream spawning migration. This is the time period when adult lamprey are most likely to orient to larval pheromones, as they are actively engaged in stream-finding and migratory movements. It was not possible to determine their gender or state of sexual maturity. However, based on the time of capture and the body morphology, it is likely that these migratory-phase individuals would not spawn for at least 9 months. After the experimentation, the lamprey were released unharmed at the original capture site.

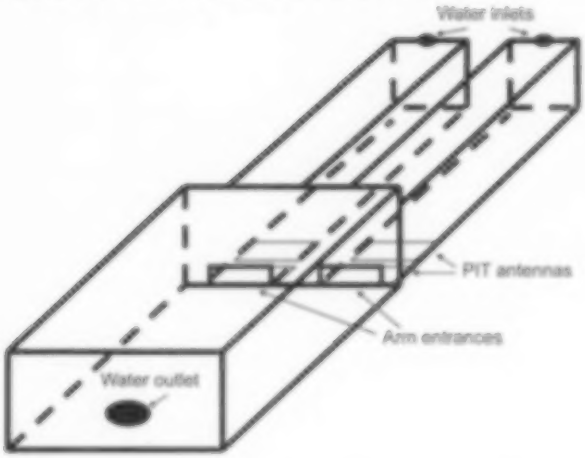
Immediately after capture, each lamprey was anesthetized, surgically implanted with a 23 mm passive integrated transponder (PIT) (Keefer et al. 2009), and acclimated to well water (10 °C) at the Big Beef Creek Research Station (BBC), Seabeck, Washington, USA. Larval lamprey odors for the behavioral trials were collected from a live culture of Pacific lamprey larvae held at the Abernathy Fish Technology Center in Longview, Washington, USA (McGree et al. 2008). Larval water was prepared by holding 20 larvae (density = 1.3 larvae per litre of Abernathy Creek water) in their rearing tanks at 8 °C without running water for 20 min. Larvae were 104–127 mm in length (mean = 115.5 mm) and weighed 1.7–3.2 g (mean = 2.4 g). Conditioned (larval) water was then collected and stored at –20 °C until later behavioural trials. Unconditioned (control) Abernathy Creek water was also collected and frozen for later use as a control.

Two-choice maze behavioral experiments

The behavioral responses of adult Pacific lampreys to water conditioned with larval lamprey odors were tested using three replicate two-choice mazes. Each maze consisted of two upstream arms (3.05 m × 0.5 m × 0.5 m tanks) flowing into a larger rectangular reservoir (3.05 m × 1.22 m × 1.22 m) with a standpipe and water outlet (Fig. 1). BBC well water was delivered to the upstream end of each arm of the maze at a rate of 12 L·min^{-1}, and water depth was maintained at 30 cm. To monitor movement in each maze, PIT tag antennas were installed under each arm (Fig. 1), and detections at each antenna were continuously recorded.

The behavioral experiments were conducted in July, when astronomical sunset at BBC was at 2110 h and sunrise was at 0520 h. Pacific lampreys displayed strongly nocturnal activity patterns, and therefore analysis was restricted to movements occurring at night. To initiate a trial, a single adult lamprey was introduced into each maze during the day, the three mazes were covered with opaque plastic, and the lampreys were allowed to acclimate for at least 3 h prior to sunset. Each lamprey remained in its maze for the duration of the trial, which involved testing over two consecutive nights; no odors were metered into the maze the first night (No Odor tests) and larval or control water was metered into the maze the second night (Odor tests). For the Odor tests, larval water

Fig. 1. Schematic diagram of a two-choice maze used in behavioral assays. Water flowed from the inlets at the end of each arm to a single outlet pipe in the reservoir. A lamprey passed from the reservoir into an arm via the arm entrance and was detected by passive integrated transponder (PIT) antennas positioned at the downstream end of the arm (shown as shaded detection areas).



was metered (5.0 mL·min^{-1}) continuously overnight into one arm of the maze (treatment arm) beginning 4–5 h before sunset. Control water from the larval rearing facility was simultaneously metered at the same rate into the other arm (control arm). At the end of the trial, the lamprey was removed, the maze was flushed with well water to remove residual odors, and the odor tubes were switched between arms for the subsequent trial to control for any inherent arm bias.

The number of entries and time spent in each arm were calculated for each maze from a continuous recording of PIT detections. Thus, a trial did not begin until a lamprey moved from the reservoir into one of the arms (Fig. 1). An entry was scored each time a lamprey moved into a given arm, and time in that arm was defined as the elapsed time from first detection to last detection at the arm entrance. The number of entries and time in each arm were tallied for each lamprey during the No Odor and Odor tests. The total number of entries and duration spent in both arms during No Odor and Odor tests were compared using a two-tailed paired *t* test.

To test for effects of larval odor, the number of entries into the treatment arm was expressed as a percentage of the total number of entries made into both arms. The resulting percentage of entries made into the treatment arm during the No Odor tests was compared with the percentage of entries made into the treatment arm during the Odor tests using a two-tailed paired *t* test. Similarly, the amount of time each lamprey spent in the treatment arm was divided by the time spent in both arms. The same statistic was used to compare the resulting percentage of time lampreys spent in the treatment arm during No Odor and Odor tests. The length of time before lampreys made their first entry into the treatment arm during the No Odor tests was compared with that time period for the Odor tests using a two-tailed paired *t* test. We used the same statistic to compare the number of entries into both arms that were made in the first hour of observation during the No Odor and Odor tests.

Isolation and analysis of bile acids

Chemicals

Methanol (HPLC grade) was obtained from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Acetonitrile and formic acid were purchased from EMD Chemicals (Gibbstown, New Jersey, USA) and Sigma-Aldrich (St. Louis, Missouri, USA), respectively.

Collection of conditioned water and extraction

For conditioned water used for extraction, Pacific lamprey larvae were collected from the Umatilla River (19–20 °C) Oregon, USA, in June using an AbP-2 electrofisher and transported to the CTUIR's Fisheries Laboratory. Larvae were divided into five groups of 500, and each group was held in a tank containing 5 L aerated well water at 16 °C for 22 h. Conditioned water was then collected for filtering and extraction. The conditioned water was filtered with 0.45 µm glass fiber filters (Whatman, Piscataway, New Jersey, USA) and loaded onto C-18 Sep-Pak cartridges (35 mL, Waters, Milford, Massachusetts, USA), followed by elution with 40 mL methanol. Methanol extracts were dried under vacuum and stored at –80 °C until analyzed.

Mass spectrometry analysis of bile acids

Samples of conditioned water extract from larval lampreys were analyzed by liquid chromatography – mass spectrometry (LC–MS) using Shimadzu HPLC system (Kyoto, Japan) interfaced with a Micromass Quattro Micro API mass spectrometer (Waters). Larval lamprey conditioned water extract (10 µL) was separated on an Atlantis C-18 column (2.1 mm × 150 mm, Waters) with a linear gradient. The mobile phase consisted of water with 0.1% formic acid (A), and acetonitrile with 0.1% formic acid (B) was applied at a flow rate of 200 µL·min^{-1}, with a linear gradient of 0%–100% B over 10 min. The effluent from the LC column was directed into the mass detector equipped with the electrospray ionization (ESI) probe. Tandem mass spectrometry (MS–MS) was performed on ions at mass-to-charge ratios (*m*/*z*) 473, 589, and 703 to confirm their chemical identity. The collision energy used was –40 eV. Nitrogen and argon gas were used for desolvation and collision, respectively. All mass spectrometry data were collected and processed using MassLynx 4.0 software (Waters).

Electro-olfactogram recordings

Adult Pacific lampreys for EOG studies were collected in July from the Bonneville Dam on the Columbia River and transported to Michigan State University's Research Containment Facility, East Lansing, Michigan, USA. Adults were held in flow-through tanks (254 L) supplied with well water (12 °C). EOG experiments were conducted during August.

The olfactory epithelia of Pacific lampreys were exposed to solutions of L-arginine (Sigma-Aldrich), used as the standard odor (Li and Sorensen 1992; Li et al. 1995), and six bile acids (ACA, PADS, PSDS, PZS, 3kACA, 3kPZS; Bridge Organics Co., Vicksburg, Michigan, USA; all >97% pure). Stock solutions of bile acids were prepared using deionized water and stored at –80 °C. A 10^{-2} mol·L^{-1} L-arginine standard stock solution was made with deionized water and stored at 4 °C. Pacific lamprey were tested for olfactory sensitivity to the six bile acids described above. EOG recording

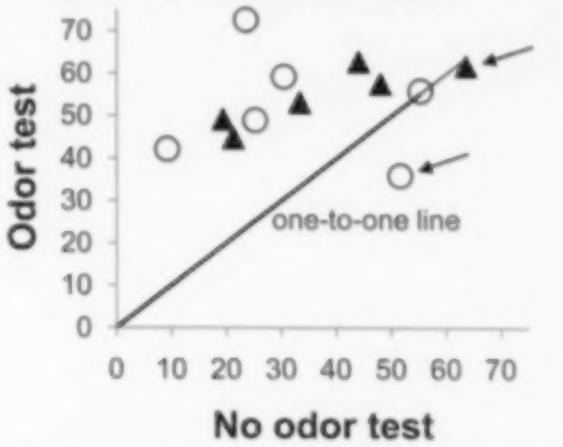
was performed as described for sea lamprey by Li et al. (1995) and Siefkes and Li (2004). Briefly, Pacific lampreys were anesthetized with an intramuscular injection of metomidate hydrochloride (3 mg·(kg body mass)$^{-1}$; Syndel, Vancouver, British Columbia, Canada), immobilized with an intramuscular injection of gallamine triethiodide (150 mg·(kg body mass)$^{-1}$; Sigma-Aldrich), and placed in a water-filled trough. The head of the lamprey remained above the water and the gills were supplied with fresh water. The olfactory lamellae were then surgically exposed and perfused with well water. Differential electrical potential between the skin surface and the sensory epithelia in response to each test stimulus was recorded using two Ag–AgCl electrodes (type EH-1S, World Precision Instruments, Sarasota, Florida, USA) filled with 3 mol·L^{-1} potassium chloride solution and bridged with 8% gelatin : 0.9% saline-filled glass capillary tubes. The recording electrode was placed between two lamellae and was adjusted to maximize the response to the L-arginine standard while minimizing the response to a blank water control when the reference electrode was placed on the skin near the naris. Electrical signals were amplified and digitized by a Power Lab (ADI Instruments, Castle Hill, Australia) and displayed on a computer. Stock solutions of bile acids were diluted in well water immediately before testing. To determine concentration–response relationships, a 10^{-4} mol·L^{-1} L-arginine standard was introduced into the olfactory epithelium of a Pacific lamprey for 5 s, and the EOG response was measured to establish a reference of electrical activity (Li et al. 1995). Next, blank water control was introduced and the response was measured to confirm the absence of response from the water supply. Increasing concentrations of bile acids starting at 10^{-15} mol·L^{-1} for PADS; 10^{-14} mol·L^{-1} for PSDS, PZS, and 3kPZS; and 10^{-13} mol·L^{-1} for ACA and 3kACA were then introduced, and the responses were measured. Measuring the response to the blank water control and L-arginine standard again at the end of the dilution series concluded each trial. The epithelium of each lamprey was allowed to recover to restore sensitivity for at least 3 min between each stimulus. A 3 min or more recovery period was chosen based on previous EOG testing experience on the sea lamprey. Additionally, each concentration of the test stimulus was assayed at least twice — a third time if sensitivity was not restored during the recovery period. EOG response magnitudes from lampreys were measured in millivolts (mV) and expressed as a percentage of the response to the L-arginine standard. Concentration–response curves for each bile acid were visually compared, and the lowest concentration at which a bile acid elicited a response larger than the blank water control (Student's t test) was considered to be the response threshold.

Results

Behavioral responses to larval odors

Lampreys were only active at night. First entries into the arms started in the evening (2133–2233, on average 59.5 min after each test was started), and last entries occurred the next morning (0430–0600). Lampreys explored both arms during the No Odor and Odor tests. During No Odor tests, the mean number of entries (both arms) was 244 (standard deviation = 102, range = 110–372), and the mean length of time spent in the arms was 82.2 min (standard deviation = 31.2 min, range = 29.0–111.4 min). During Odor tests, the mean number of entries was 237 (standard deviation = 50, range = 158–293), and the mean length of time spent in the arms was 102.7 min (standard deviation = 14.3 min, range = 90.7–125.7 min). There was no significant difference in these metrics, total number of entries (P = 0.82, df = 5, t = 0.24), or total time spent in both arms (P = 0.14, df = 5, t = 1.74), during the No Odor and Odor tests.

Fig. 2. The percentage of time each lamprey spent (open circles) in the treatment arm during the No Odor tests and during the Odor tests. Data for the percentage of entries made into the treatment arm during No Odor and Odor tests are also given for each fish (dark triangles). For example, the only lamprey that was not attracted by larval odor (indicated by arrow) spent 52% of its time in the treatment arm during the No Odor tests and 36% of its time in the treatment arm during the Odor tests. During the No Odor tests, this fish made 64% of its entries into the treatment arm, and during the Odor tests it made 62% of its entries into the treatment arm.



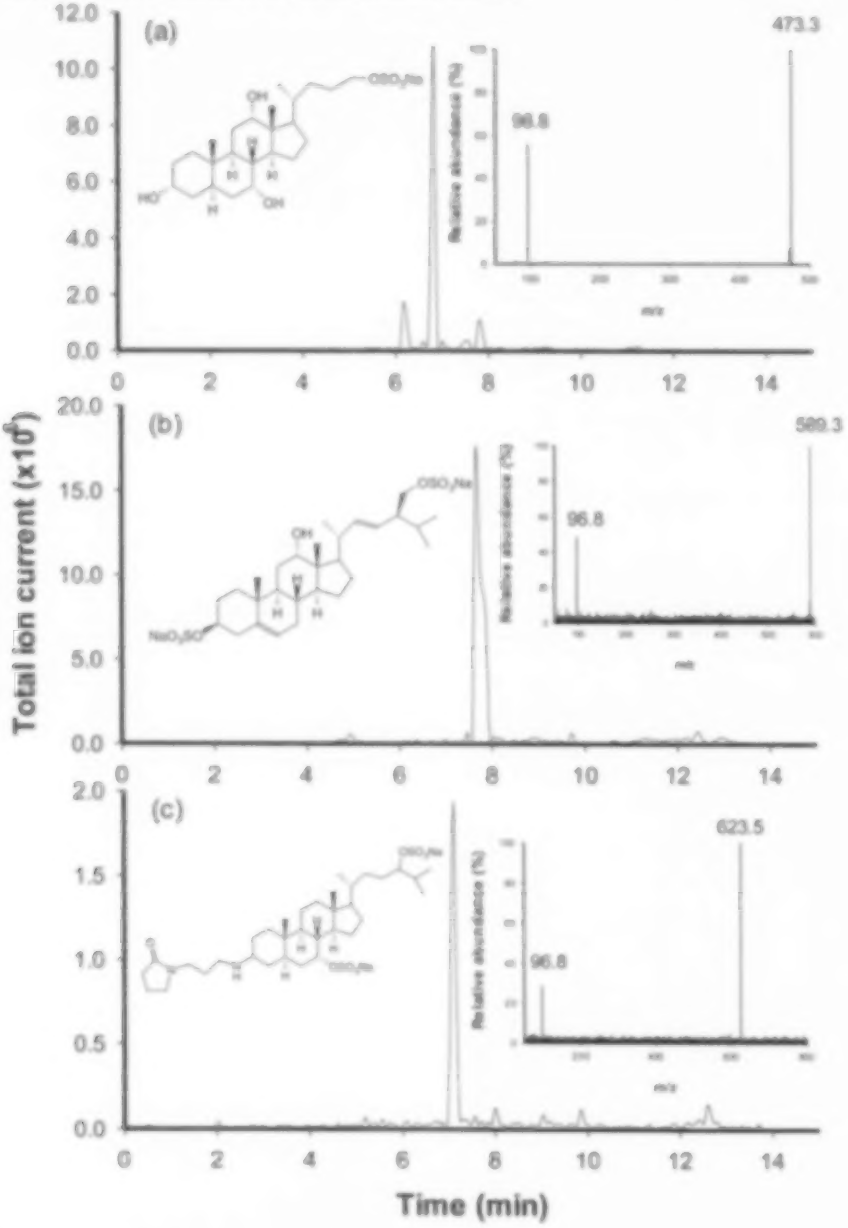
The lampreys showed a significantly higher (P = 0.01, df = 5, t = 3.63) percentage of entries into the treatment arm during the Odor tests (mean = 55%, standard deviation = 17%) than during the No Odor tests (mean = 38%, standard deviation = 7%). Only one of the six animals was not attracted by the larval odor (Fig. 2). During the first hour of the Odor tests, lampreys made more entries into both arms than during the first hour of the No Odor tests (P = 0.03, df = 5, t = 2.91). However, there was no indication that lampreys entered the treatment arm earlier during the Odor tests than during the No Odor tests (P = 0.13, df = 5, t = 1.82).

Lampreys also spent a significantly higher (P = 0.09, df = 5, t = 2.08) percentage of their time in the treatment arm during the Odor tests (mean = 53%, standard deviation = 13%) than during the No Odor tests (mean = 33%, standard deviation = 18%) (Fig. 2). One lamprey spent equal amounts of time in the treatment arm during both tests, and one spent less time in the treatment arm during the Odor tests. The remaining four fish showed clear preference for the treatment arm when larval odor was present (Fig. 2).

Extraction and identification of larval bile acids

LC–MS analyses of larval conditioned water extracts revealed that the larval Pacific lamprey could release conju-

Fig. 3. Identification of bile acid compounds from larval Pacific lamprey. Liquid chromatography – mass spectrometry (LC–MS) analyses were performed at negative mode on the extract of conditioned water with larval Pacific lamprey. Multiple reaction monitoring (MRM) analyses of the extract revealed the presence of three major bile acid compounds such as petromyzonol sulfate (*m/z* 473.3 > 96.8), petromyzosterol disulfate (*m/z* 589.3 > 96.8), and petromyzonamine disulfate (*m/z* 703.3 > 96.8), respectively. Fragmentation patterns and chemical structures of each compound are represented in the inset. (*a*) LC–MS identification of petromyzonol sulfate, (*b*) LC–MS identification of petromyzosterol disulfate, (*c*) LC–MS identification of petromyzonamine disulfate.



gated bile acids that were known to act as migratory pheromones in sea lamprey. HPLC separation followed by ESI–MS detection of the extract identified major peaks at *m/z* 473.30, 589.36, and 703.34 (Fig. 3). To confirm their identities, an ESI–MS–MS analysis was performed for each peak to obtain fragmentation patterns (Fig. 3, inset) that matched with those for each compound as already described in an earlier study (Sorensen et al. 2005). Signature daughter ions originating from ionization of HSO_4 were observed at *m/z* 96.8 for PZS, PSDS, and PADS (Fig. 3, insets). PADS produced an ionized peak at *m/z* 623.5, indicating loss of SO_3 (–80) from PADS (Fig. 3*c*, inset). In addition, a trace

amount of ACA and 3kPZS was found to be present in the extract of larval lamprey (data not shown).

Electro-olfactogram experiments of the identified bile acids

All compounds tested stimulated the olfactory epithelium of Pacific lampreys (Fig. 4). The mean response to the L-arginine standard was 0.247 ± 0.007 mV (mean ± standard error, SE). Concentration–response relationships of the six bile acids were plotted as percentages of the L-arginine standard (Fig. 5). All responses to bile acids ranged from 1% to 1050%, and the blank water control elicited a mean response of 5.4% ± 0.7% (mean ± SE). The 3kACA ($N = 5$), ACA ($N = 5$), PADS ($N = 5$), and PSDS ($N = 4$) had similar concentration–response curves that were more linear in shape (Figs. 5*a*, 5*b*). The response threshold for 3kACA was 10^{-10} mol·L^{-1} (10.9% ± 3%, mean ± SE; Student's t test, $P < 0.02$; Figs. 4 and 5*a*), for ACA was 10^{-9} mol·L^{-1} (50.9% ± 6.3%; $P < 0.0001$; Figs. 4 and 5*a*), for PADS was 10^{-13} mol·L^{-1} (64.7% ± 12%; $P < 0.0001$; Figs. 4 and 5*b*), and for PSDS was 10^{-11} mol·L^{-1} (19% ± 2.3%; $P = 0.05$; Figs. 4 and 5*b*). The 3kPZS and PZS had similar concentration–response curves that were exponential in shape with steep slopes between 10^{-10} and 10^{-5} mol·L^{-1}. The response threshold for 3kPZS and PZS was 10^{-14} mol·L^{-1} (23.1% ± 5.5%; $P < 0.02$; and 15.%7 ± 2.6%; $P < 0.02$, respectively; Figs. 4 and 5*c*). The concentration–response curves for 3kACA, ACA, PADS, and PSDS were shallower than the curves generated using 3kPZS and PZS.

Discussion

With this study we demonstrate a chemical communication system in Pacific lamprey. During behavioral assays, adult Pacific lampreys exhibited strong attraction to odors released by larval conspecifics. We then identified the bile acid compounds released by larval Pacific lamprey using solid phase extraction of the conditioned water followed by LC–MS analysis. Tandem mass spectrometry with ESI on the conditioned water extract from larval Pacific lamprey revealed that three sulfated bile acids (PZS, PADS, and PSDS) were present and that their chemical structures were identical to the bile acid compounds that are known to function as migratory pheromones in the sea lamprey. Finally, we demonstrated that the Pacific lamprey olfactory system can detect the sulfated bile acid compounds at concentrations in the picomolar range. Taken together, our findings strongly suggest that Pacific lamprey use a chemical communication system for orientation and navigation during their spawning migration.

Our behavioral assays indicated that adult lampreys were attracted to water conditioned with larval conspecifics. Similar behavioral assays with Y mazes have been used to document the attraction of both adult river (*Lampetra fluviatilis*) and sea lamprey to larval lamprey pheromones (Bjerselius et al. 2000; Vrieze and Sorensen 2001; Gaudron and Lucas 2006). Moreover, this method has been used to examine responses of adult female sea lamprey to male sex pheromones (Li et al. 2002; Siefkes et al. 2003; Johnson et al. 2006). It is possible that the adult Pacific lamprey used in this study were exhibiting a generalized form of conspecific attraction, rather than responses to a migratory cue. To test for this, additional assays of this kind are needed using life stages that are not in the migratory phase (i.e., parasitic phase or spawning phase lamprey).

In previous behavioral studies, lamprey movements were monitored over relatively short time periods (minutes to hours) and often with only a single pulse of odor. Use of the PIT tag technology in our experiments allowed the observation of lamprey behavior over the course of several nights without disrupting lamprey movements. In addition, we were able to examine behavior during nights of continuous exposure to larval odors. During these Odor tests, lampreys made more entries into the treatment arm and spent more time there than during No Odor tests, clearly demonstrating a preference for water conditioned with larval lampreys. However, in our completely volitional experiments, the lampreys did not initiate movements into the treatment arm any sooner when larval odor was present. This result suggests that diel activity rhythms supersede olfactory stimuli at this life stage. Moreover, it is possible that short-term experiments would not have captured the complex searching behaviors exhibited by these fish.

The chemical identities of the putative pheromone compounds in Pacific lamprey were studied using ESI–LC–MS analysis, as proven useful in Sorensen et al. (2005). Each compound was separated on a C-18 column followed by an MS detection method and an MRM method (multiple reaction monitoring), which is widely used to identify and quantify bile acid compounds (Griffiths and Sjovall 2010). These larval compounds all possess at least one sulfate group, which can give off a typical signal at m/z 97 when fragmented by MS–MS. In this study, we have used pairs of m/z 473 > 97 for PZS, m/z 589 > 97 for PSDS, and m/z 703 > 97 for PADS to monitor their elution on a C-18 column. This MRM separation combined with MS–MS data showing fragmentation patterns of each compound clearly demonstrates the presence of the three bile acid compounds in the conditioned water with larval Pacific lamprey. Identification of the three bile acid compounds that were previously characterized in the sea lamprey indicates involvement of these compounds as chemical cues modifying Pacific lamprey behavior.

EOG recordings have been actively used to characterize olfactory mechanisms in fish (Scott and Scott-Johnson 2002). EOG responses in this study tended to be lower than in previously reported work on both sea lamprey (Sorensen et al. 2005) and Pacific lamprey (Robinson et al. 2009) and may have resulted from a number of factors, including maturation state, time of year, overall health, holding conditions, and experimental equipment. Variation in response magnitude is inherent in EOG studies and is accounted for by comparing responses of test compounds with responses from a standard.

Concentration–response curves generated for ACA, 3kACA, PZS, and 3kPZS in this study were similar in shape to those generated in sea lamprey (Siefkes and Li 2004). Concentration–response curves generated for PADS and PSDS were much different than those generated in sea lamprey (Sorensen et al. 2005) and were atypical of concentration–response curves seen for lampreys (Siefkes and Li 2004; Sorensen et al. 2005; Robinson et al. 2009). Importantly, during our EOG experiments, PADS and PSDS were typically tested before or after the other bile acids on

Fig. 4. Representative electro-olfactogram (EOG) responses of adult Pacific lampreys to the 10^{-4} mol·L^{-1} L-arginine standard (Std), blank water (Blk), and concentrations of allocholic acid (ACA), 3-keto allocholic acid (3kACA), petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), petromyzonol sulfate (PZS), and 3-keto petromyzonol sulfate (3kPZS). NT denotes that concentrations were not tested.

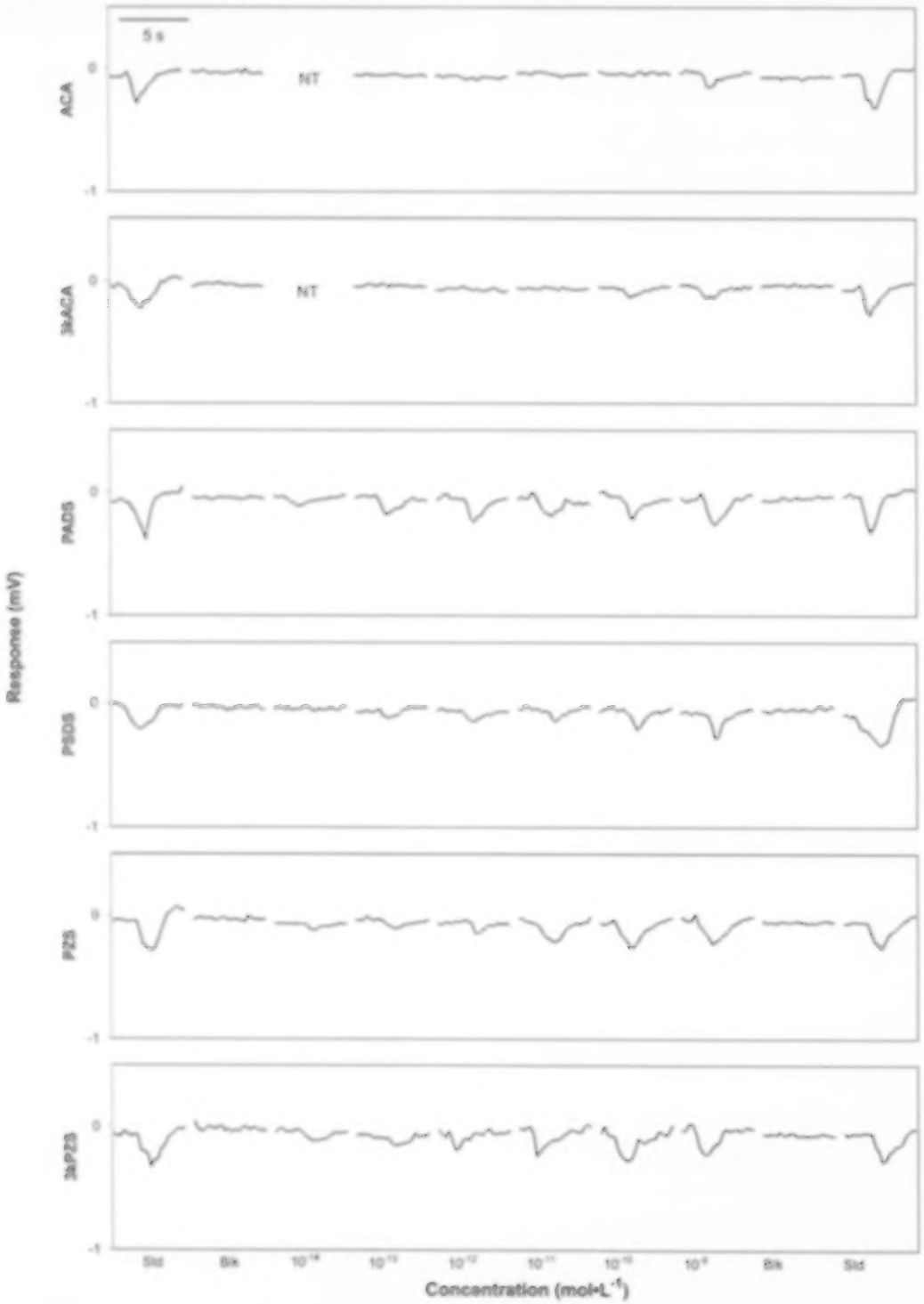
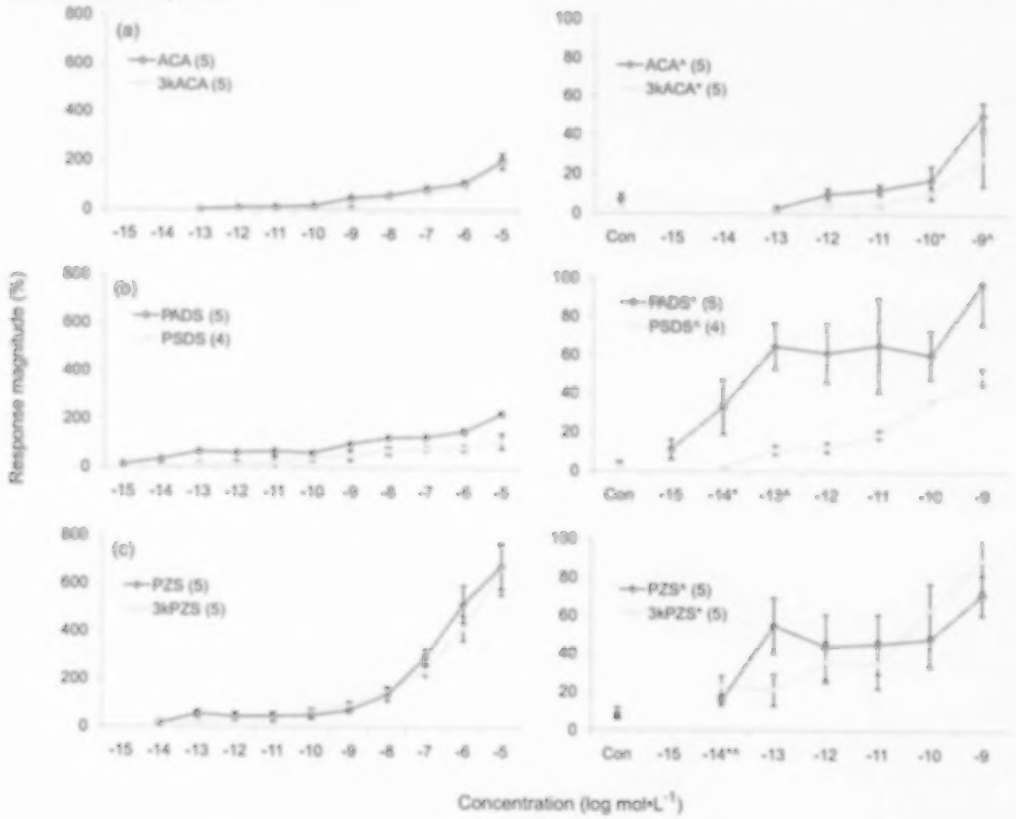


Fig. 5. Semilogarithmic plots of electro-olfactogram (EOG) concentration–responses to (*a*) allocholic acid (ACA) and 3-keto allocholic acid (3kACA), (*b*) petromyzonamine disulfate (PADS) and petromyzosterol disulfate (PSDS), and (*c*) petromyzonol sulfate (PZS) and 3-keto petromyzonol sulfate (3kPZS). Plots on the right are expanded views to show detection thresholds and to visually compare bile acid responses to a blank water control (Con). The detection threshold for a given odor, indicated by asterisks (*) and carrots (^), is the lowest concentration that elicits a response significantly greater than the blank water control (Student's *t* test, $P < 0.05$). Mean response magnitudes are presented as a percentage of the response elicited by a 10^{-4} mol·L^{-1} L-arginine standard solution. Vertical bars represent one standard error. Numbers by the abbreviations indicate sample size.



the same test subject, indicating that condition of the test subject was not likely responsible for the different results observed between Pacific and sea lampreys. The difference, however, could possibly be explained by interspecies differences in olfactory receptors and indicate that PADS and PSDS do not function as a pheromone in the Pacific lamprey.

The detection thresholds for PZS and 3kPZS observed in this study were statistically much lower than those previously reported for Pacific lamprey (Robinson et al. 2009). The first concentration tested (10^{-14} mol·L^{-1}), however, was significantly different than the blank control, and responses did not increase until 10^{-10} mol·L^{-1}. These results may indicate a problem with the EOG recordings or odorant solutions at lower concentrations. Therefore, the detection thresholds for PZS and 3kPZS were likely three orders of magnitude greater (10^{-11} mol·L^{-1}) than the statistics indicated in this study.

Nevertheless, our EOG data indicated that Pacific lamprey are able to smell these bile acid compounds, which is a prerequisite for compounds to qualify as chemical cues. The Pacific lamprey olfactory system responded more strongly to PZS and 3kPZS than to other sulfated compounds such as PADS and PSDS and free bile acids such as ACA and 3kACA. The detection threshold for the bile acid compounds, however, might be a more important factor than the strength of the responses in determining whether they are functional or not. As in the sea lamprey, a mixture of the three compounds — PZS, PADS, and PSDS — may be needed to act as migratory pheromone and elicit behavioral responses (Sorensen et al. 2005; Fine and Sorensen 2008). Additional EOG studies are required to further characterize the olfactory responses of the Pacific lamprey to compounds released by conspecifics and identify additional pheromone components that may be needed to elicit the full suite of behavioral responses. In addition, cross-adaptation studies to characterize the number of olfactory receptor mechanisms mediating responses to known ligands can further our understanding of olfactory mechanisms mediated by the bile acid compounds in the Pacific lamprey, as evidenced by an earlier study in the sea lamprey (Siefkes and Li 2004).

Chemical communication mechanisms in insects have been extensively studied and have provided practical tools to manipulate insect populations in the agricultural setting (Cardé

and Minks 1996). However, understanding of chemical communication in fishes has been slow to emerge (Sorensen et al. 2005; Stacey and Sorensen 2009; Stacey 2010) because of difficulties associated with working in aquatic environments. Considering that fish rely heavily on olfaction to find food, avoid predators, select mates, and coordinate reproductive behavior, a better understanding of fish behavior mediated by chemical cues is needed. Such findings may lead to strategies for modifying migratory and mating behavior, leading to practical management tools for fish populations of interest. This has been well demonstrated in the nonindigenous sea lamprey in the Great Lakes. The mating pheromone in the sea lamprey currently is being developed as a management tool to help control the sea lamprey population by capturing ovulatory females using the synthetic mating pheromone, 3kPZS (Johnson *et al.* 2005, 2006, 2009). In addition, chemosensory mechanisms that guide migrating lamprey to choose a stream are being considered for lamprey population management (Li et al. 2003; Sorensen and Vrieze 2003).

Our findings suggest a biological role for these putative pheromones in modifying migration behavior of Pacific lamprey adults. This is particularly interesting in light of the fact that Pacific lamprey must often execute spawning migrations over greater distances (hundreds of kilometres) and over longer time periods (1–2 years) than sea lamprey (Keefer et al. 2009). In the present study, we demonstrated behavioral responses to chemical cues that may mediate adult Pacific lamprey migratory behavior, and we chemically identified putative pheromones released by larval Pacific lamprey that triggered electrophysiological responses in the adults. Further research on Pacific lamprey chemical communication systems is warranted and may open a path to novel strategies for Pacific lamprey recovery.

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References

Bjerselius, R., Li, W., Teeter, J.H., Seelye, J.G., Johnsen, P.B., Maniak, P.J., Grant, G.C., Polkinghorne, C.N., and Sorensen, P.W. 2000. Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone. Can. J. Fish. Aquat. Sci. **57**(3): 557–569. doi:10.1139/f99-290.

Cardé, R.T., and Minks, A.K. 1996. Insect pheromone research: new directions. Chapman & Hall, New York.

Close, D.A., Fitzpatrick, M.S., and Li, H.W. 2002. The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries, **27**(7): 19–25. doi:10.1577/1548-8446(2002)027<0019:TEACIO>2.0.CO;2.

Close, D.A., Currens, K.P., Jackson, A., Wildbill, A.J., Hansen, J., Bronson, P., and Aronsuu, K. 2009. Lessons from the reintroduction of a noncharismatic, migratory fish: Pacific lamprey in the Upper Umatilla River, Oregon. *In* Biology, management, and conservation of lampreys in North America. *Edited by* L.R. Brown, S.D. Chase, M. Mesa, R. Beamish, and P.B. Moyle. American Fisheries Society, Symposium 72, Bethesda, Md. pp. 233–253.

Fine, J.M., and Sorensen, P.W. 2008. Isolation and biological activity of the multicomponent sea lamprey migratory pheromone. J. Chem. Ecol. **34**(10): 1259–1267. doi:10.1007/s10886-008-9535-y. PMID:18784958.

Fine, J.M., Vrieze, L.A., and Sorensen, P.W. 2004. Evidence that petromyzontid lampreys employ a common migratory pheromone that is partially comprised of bile acids. J. Chem. Ecol. **30**(11): 2091–2110. doi:10.1023/B:JOEC.0000048776.16091.b1. PMID:15672658.

Gaudron, S.M., and Lucas, M.C. 2006. First evidence of attraction of adult river lamprey in the migratory phase to larval odour. J. Fish Biol. **68**(2): 640–644. doi:10.1111/j.0022-1112.2006.00937.x.

Griffiths, W.J., and Sjovall, J. 2010. Analytical strategies for characterization of bile acid and oxysterol metabolomes. Biochem. Biophys. Res. Commun. **396**(1): 80–84. doi:10.1016/j.bbrc.2010.02.149. PMID:20494115.

Johnson, N.S., Siefkes, M.J., and Li, W. 2005. Capture of ovulating female sea lampreys in traps baited with spermiating male sea lampreys. N. Am. J. Fish. Manage. **25**(1): 67–72. doi:10.1577/M03-226.1.

Johnson, N.S., Luehring, M.A., Siefkes, M.J., and Li, W. 2006. Mating pheromone reception and induced behavior in ovulating female sea lampreys. N. Am. J. Fish. Manage. **26**(1): 88–96. doi:10.1577/M05-018.1.

Johnson, N.S., Yun, S.-S., Thompson, H.T., Brant, C.O., and Li, W. 2009. A synthesized pheromone induces upstream movement in female sea lamprey and summons them into traps. Proc. Natl. Acad. Sci. U.S.A. **106**(4): 1021–1026. doi:10.1073/pnas.0808530106. PMID:19164592.

Keefer, M.L., Moser, M.L., Boggs, C.Y., Daigle, W.R., and Peery, C.A. 2009. Variability in migration timing of adult Pacific lamprey (*Lampetra tridentata*) in the Columbia River, USA. Environ. Biol. Fishes, **85**(3): 253–264. doi:10.1007/s10641-009-9490-7.

Kostow, K. 2002. Oregon lampreys: natural history status and analysis of management issues [online]. Oregon Department of Fish and Wildlife, Portland, Ore. Available from http://nrimp.dfw.state.or.us/web%20stores/data%20libraries/files/ODFW/ODFW_509_2_lampreys2.pdf.

Li, W., and Sorensen, P.W. 1992. The olfactory sensitivity of sea lamprey to amino acids is specifically restricted to arginine. Chem. Senses, **17**: 658.

Li, W., Sorensen, P.W., and Gallaher, D.D. 1995. The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is acutely and specifically sensitive to unique bile acids released by conspecific larvae. J. Gen. Physiol. **105**: 569–587. doi:10.1085/jgp.105.5.569. PMID:7658193.

Li, W., Scott, A.P., Siefkes, M.J., Yan, H., Liu, Q., Yun, S.-S., and Gage, D.A. 2002. Bile acid secreted by male sea lamprey that acts as a sex pheromone. Science, **296**(5565): 138–141. doi:10.1126/science.1067797. PMID:11935026.

Li, W.M., Siefkes, M.J., Scott, A.P., and Teeter, J.H. 2003. Sex pheromone communication in the sea lamprey: implications for

integrated management. J. Great Lakes Res. **29**: 85–94. doi:10.1016/S0380-1330(03)70479-1.

McGree, M., Whitesel, A.A., and Stone, J. 2008. Larval metamorphosis of individual Pacific lamprey reared in captivity. Trans. Am. Fish. Soc. **137**(6): 1866–1878. doi:10.1577/T07-206.1.

ODFW. 2006. Oregon native fish status report [online]. Oregon Department of Fish and Wildlife, Salem, Ore. Available from http://www.dfw.state.or.us/fish/ONFSR/report.asp.

Robinson, C.T., Sorensen, P.W., Bayer, J.M., and Seelye, J.G. 2009. Olfactory sensitivity of Pacific lampreys to lamprey bile acids. Trans. Am. Fish. Soc. **138**(1): 144–152. doi:10.1577/T07-233.1.

Scott, J.W., and Scott-Johnson, P.E. 2002. The electroolfactogram: a review of its history and uses. Microsc. Res. Tech. **58**(3): 152–160. doi:10.1002/jemt.10133. PMID:12203693.

Siefkes, M.J., and Li, W. 2004. Electrophysiological evidence for detection and discrimination of pheromonal bile acids by the olfactory epithelium of female sea lamprey (*Petromyzon marinus*). J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. **190**(3): 193–199. doi:10.1007/s00359-003-0484-1. PMID:14689221.

Siefkes, M.J., Winterstein, S., and Li, W. 2003. Chemosterilization of male sea lampreys (*Petromyzon marinus*) does not affect sex pheromone release. Can. J. Fish. Aquat. Sci. **70**: 1037–1045. doi:10.1139/f02-169.

Sorensen, P.W., and Vrieze, L.A. 2003. The chemical ecology and potential application of the sea lamprey migratory pheromone. J. Great Lakes Res. **29**: 66–84. doi:10.1016/S0380-1330(03)70478-X.

Sorensen, P.W., Fine, J.M., Dvornikovs, V., Jeffrey, C.S., Shao, F., Wang, J., Vrieze, L.A., Anderson, K.R., and Hoye, T.R. 2005. Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. Nat. Chem. Biol. **1**(6): 324–328. doi:10.1038/nchembio739. PMID:16408070.

Stacey, N.E. 2010. Hormonally-derived sex pheromones in fish. *In* Hormones and reproduction in vertebrates. Vol. 1. Fishes. *Edited by* D.O. Norris and K.H. Lopez. Academic Press, New York. pp. 169–192.

Stacey, N.E., and Sorensen, P.W. 2009. Fish hormonal pheromones. *In* Hormones, brain, and behavior. 2nd ed. Vol. 2. *Edited by* D.W. Pfaff, A.P. Arnold, A.M. Etgen, S.E. Fahrbach, and R.T. Rubin. Elsevier, San Diego, Calif. pp. 639–681.

Vrieze, L.A., and Sorensen, P.W. 2001. Laboratory assessment of the role of a larval pheromone and natural stream odor in spawning stream localization by migratory sea lamprey (*Petromyzon marinus*). Can. J. Fish. Aquat. Sci. **58**(12): 2374–2385. doi:10.1139/f01-179.

Yun, S.-S., Scott, A.P., Bayer, J.M., Seelye, J.G., Close, D.A., and Li, W. 2003. HPLC and ELISA analyses of larval bile acids from Pacific and western brook lampreys. Steroids, **68**(6): 515–523. doi:10.1016/S0039-128X(03)00088-6. PMID:12906936.

PERSPECTIVE / PERSPECTIVE

Direct and indirect estimates of the productive capacity of fish habitat under Canada's Policy for the Management of Fish Habitat: where have we been, where are we now, and where are we going?

Charles K. Minns, Robert G. Randall, Karen E. Smokorowski, Keith D. Clarke, Antonio Vélez-Espino, Robert S. Gregory, Simon Courtenay, and Patrice LeBlanc

Abstract: No net loss of productive capacity (PC) of fish habitat has been the central concept guiding Canadian fish habitat management policy since 1986. The purpose of this paper is to describe the concept of PC, to review the history and application of the fish habitat management policy in Canada, and to provide a critical review of the range of potential approaches to estimating PC. The approaches were grouped by their central focus: habitat, individual, population, and community–ecosystem. A set of case studies is used to illustrate the use of some approaches drawn from freshwater and marine contexts. Ten components to assessing no net loss of PC were developed and used in the review of approaches for evaluating potential limitations. The review also highlighted the likely future direction of method development, with increasing emphasis on dynamic models integrating population responses to habitat supply characteristics. More work needs to be done to turn research-based metrics of PC into practical operational management assessment tools and to better quantify the link between habitat structure and function and fisheries productivity. The evolving approaches to measure PC reinforce the ties that fish habitat management has to the emerging practices in ecosystem-based management.

Résumé : La prévention de la perte nette de la capacité de production de l'habitat des poissons constitue le concept central qui régit la politique canadienne de gestion des habitats de poissons depuis 1986. L'objectif de notre travail est de décrire le concept de la capacité de production, de récapituler l'histoire et l'application de la gestion des habitats de poissons au Canada et présenter une revue critique de la gamme des approches potentielles pour estimer la capacité de production. Ces approches ont été regroupées en fonction de leur cible principale, soit l'habitat, l'individu, la population et la communauté et l'écosystème. Nous présentons une série d'études de cas pour illustrer l'utilisation de quelques approches tirées des contextes marins et d'eau douce. Nous mettons au point dix composantes pour évaluer la prévention de la perte nette de la capacité de production et les utilisons dans notre revue des approches pour évaluer les limitations potentielles. Notre revue souligne aussi la direction future vraisemblable de la mise au point de méthodes, avec une emphase croissance sur les modè-

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les dynamiques qui intègrent les réactions des populations aux caractéristiques des apports des habitats. Il est nécessaire de faire plus de travail pour traduire les métriques de la capacité de production basées sur la recherche en outils d'exécution pratiques d'évaluation de la gestion et pour mieux mesurer le lien entre la structure et le fonctionnement des habitats et la productivité des pêches. Les approches en cours pour mesurer la capacité de production renforcent les liens qui existent entre la gestion des habitats des poissons et les pratiques émergentes dans la gestion basée sur les écosystèmes.

[Traduit par la Rédaction]

Introduction

Canada's freshwater and marine fish species and fish habitats have a vital role in the economic prosperity of Canada, the biological diversity of Canada, and the lives of Canadians. They provide an important source of food and enjoyment, contributing many jobs and ~$12 billion to the economy (www.dfo-mpo.gc.ca/stats/FastFacts_09-eng.pdf). They provide food and a living for aboriginal peoples and are a critical part of their culture. They play an important role in the economy, life, and well-being of rural, coastal, and northern communities. They are a primary feature of the biological diversity, integrity, health, and productivity of aquatic ecosystems and represent a powerful symbol and strong indicator of the quality of Canada's natural environment. However, they are under significant pressure from unprecedented increases in the diversity and intensity of uses (Chu et al. 2003), while public concerns and scrutiny are escalating in parallel (Schindler 2001). Thus, how fragmentation and losses of fish habitat are managed in Canada and, in particular, how science-based evidence and methods are used to guide development decisions affecting fish habitat are important questions.

The purpose here is to provide a review of potential approaches to the measurement of productive capacity (PC), which is a central concept in fish habitat management in Canada. For context, we begin with an overview of fish habitat management in Canada and of definitions of PC and related concepts. Next, we present an extensive review of potential measures of PC, recognizing that in most habitat management contexts, direct measurement of PC is not feasible and that surrogate indicators, both partial and complete, will have a central role. Then we use some case studies to expose some of the challenges faced when trying to apply these approaches.

Fish habitat management in Canada

Under Canada's *Constitution Act* (1982), the federal government is assigned legislative responsibilities for "sea-coast and inland fisheries," whereby Canada's Parliament establishes laws for the conservation and protection of its fisheries resources, inclusive of fish habitats. Fisheries and Oceans Canada (DFO) is responsible for the administration of the *Fisheries Act* (FA), the *Oceans Act* (OA), and the aquatic species part of the *Species at Risk Act* (SARA). Canada's *Fisheries Act* of 1868 established the legal framework governing sea coast and inland fisheries. The FA also includes authorities for regulating a variety of impacts to fishes and fish habitat known as the "habitat protection and pollution prevention" provisions. Environment Canada (EC) has the delegated responsibility for the administration of Section 36 of the FA for regulating water pollution through a 1985 Memorandum of Understanding (MOU). Provinces, through delegation of inland fisheries and constitutional responsibilities for the management of natural resources, deliver a range of programs related to the management of freshwaters complementing those of the federal government for the management of fish habitat. The key habitat protection and pollution prevention provisions in the FA are Section 32, which prohibits destruction of fish by other means than fishing, Section 35, which prohibits harmful alteration, disruption, or destruction (HADD) of fish habitat, and Section 36, which prohibits the deposit of deleterious substances in water frequented by fishes (for more details, see www.dfo-mpo.gc.ca/habitat). DFO conducts environmental assessments (EAs) under the *Canadian Environmental Assessment Act* (CEAA) or other EA processes in the North, meets requirements under the SARA and duty for Aboriginal consultations prior to making regulatory decisions under the FA, and coordinates with EC on delivery of the pollution prevention provisions of the FA. As a result, many consider the FA to be one of the strongest environmental laws in Canada.

In 1986, DFO introduced the Policy for the Management of Fish Habitat (Fisheries and Oceans Canada (DFO) 1986) as a comprehensive framework for the administration of the habitat protection and pollution prevention provisions of the FA. The policy includes (*i*) an objective, net gain of PC of fish habitat, (*ii*) three goals, conservation, restoration, and development of fish habitat, and (*iii*) a guiding principle, no net loss (NNL) of PC of fish habitat, to support the conservation goal. The policy also includes (*i*) procedures for applying the NNL principle, (*ii*) a framework on integrated planning for habitat management, and (*iii*) eight implementation strategies.

DFO's Habitat Management Program (HMP) administers the habitat protection provisions using policy tools, including the risk management framework, habitat compensation (replacement of natural habitat) guidelines, operational statements, and procedural protocols (Goodchild 2004). In practice, HMP reviews development proposals referred (referrals) to DFO to determine if they will result in adverse impacts to fishes and fish habitat, negotiates to avoid any adverse impacts, or considers authorizations under the FA. Where an authorization is issued, it negotiates a compensation plan designed to offset the loss of fish habitat and to monitor its implementation. Reviewing referrals, habitat management staff apply a risk management framework (RMF) with three components: (*i*) aquatic effects assessment to identify the potential effects to fishes and fish habitat using pathways of effects (PoE) diagrams (www.dfo-mpo.gc.ca/habitat/what-quoi/2-evalh-eng.htm); (*ii*) risk assessment to determine the level of risk posed by residual effects; and (*iii*) risk management in which risk level (low, medium, high) is characterized and guides practitioners to tools that can be used to best manage the risk.

DFO receives and reviews numerous FA referrals and CEAA environmental screenings each year. All these fish habitat management activities must be supported by science-based concepts and techniques that address the measurement of PC and how it responds to habitat changes.

Productive capacity and related concepts in fisheries science

The concept of PC is central to DFO's fish habitat management activities and is defined as "the maximum natural capability of habitats to produce healthy fish, safe for human consumption, or to support or produce aquatic organisms upon which fish depend" (DFO 1986). PC is part of a growing lexicon of concepts that are the currency of modern attempts at ecosystem-based management, including habitats, physical and chemical processes, biodiversity, and productivity of the species and communities (Gavaris 2009). Fish production is usually defined as the product of average biomass and specific growth rate ($kg \cdot ha^{-1} \cdot year^{-1}$) (Randall and Minns 2000; Randall 2003), and for a defined area of habitat, PC is "the sum of all production accrued by all stock during the time they spend any part of their life history in that area" (Minns 1997, p. 2464).

In fisheries science, PC is tied to the concept of maximum sustained yield (MSY) as the yield taken at the maximum rate of production (Rosenberg et al. 1993; Randall 2003), although various risk factors have largely rendered MSY unsuitable for regulating exploitation of fisheries resources (Larkin 1996). A related natural resource management term, carrying capacity (CC), defined as the equilibrium maximum abundance of a population in the absence of exploitation, has also been greatly criticised (Sayre 2008). For fisheries scientists, these key terms (PC, CC, and MSY) are more familiarly linked via the terms in simple population growth and stock biomass models long used in fisheries (e.g., Kashiwai 1995; Williams 1999; Quinn and Collie 2005). In the simplest models, MSY is a function of maximum instantaneous growth rate and the equilibrium unexploited biomass, or CC. Thus PC, which is tied to the quality and quantity of suitable habitats, fits easily within the main body of fisheries science (Randall 2003). The scientific foundations of PC are sound, making it suitable as a basis for assessing potential fisheries value of habitats.

Since the introduction of the Policy for the Management of Fish Habitat in Canada (DFO 1986), considerable scientific research effort has been devoted to rendering the concept of PC in quantitative tools and methodologies to apply the principle of NNL of PC. By definition, net change of PC should be assessed in units of production (mass per area per time). However, the knowledge gained in ecosystem science in recent decades has made it clear that PC must also take account of the many structural (e.g., biodiversity and habitat) and functional (e.g., energy and material flows) ecosystem features essential for sustained natural productivity. Many surrogate and partial measures of PC fall short of the ideal but nonetheless have contributed to the evolution of a scientific framework for the implementation of DFO's Policy for the Management of Fish Habitat (DFO 1986). Indeed, most fish habitat management in Canada is based on the use of PC purely as an abstract implicit concept and not a concrete measurable quantity.

Our purpose here is to review and evaluate progress in fish habitat science as indicated by the development of pieces of science that are gradually advancing the potential to shift the role of PC from the abstract to the concrete. Approaches for measuring PC and for assessing net change of PC are examined in a series of categories beginning with those relying almost entirely on measurements of habitat characteristics, through those linking habitat features with individual, population, and community–ecosystem fish characteristics. Case studies are presented to illustrate the main features of several approaches. Trends, degree of progress, and emerging directions of the science are appraised and suggestions for future priorities are identified. The potential for using PC and NNL as central features in ecosystem-based management of fish habitats is examined.

To provide context and a point of reference for this review, 10 key components for assessing NNL of PC were identified (Table 1). Components 1–3 establish the frames of reference for an assessment, along with the relative values assigned to various fish components and the areas of habitats by type. Components 4–6 are where measurements and units are specified and applied to estimate productivity capacity, albeit in a surrogate form in many approaches. Components 7–8 are where alternate development configurations are considered to minimize or avoid a loss, with attention to uncertainties, risks, and timing (Minns and Moore 2003; Minns 2006). Components 9–10 allow for the potential incompleteness of the net change assessment with regard to additional habitat and biotic cofactors beyond the physical aspects of fish habitats. For example, the abundant presence of a non-fish invasive species or persistent organic contamination of bottom substrates and the food web may preclude attainment of a preferred level of fish productivity.

Approaches

Measures of PC have been developed using a broad array of approaches. These approaches were classified into four groups: habitat, individual, population, and community–ecosystem. Within these groups, both assessment methods and models were examined. The potential role of methods involving lower trophic levels was not considered, although many methodologies do use non-fish biota as a basis for evaluating the status of aquatic habitat with composition and abundance data (Barbour et al. 1995; Niemi and McDonald 2004; Bonada et al. 2006) or with production data (Downing et al. 1990; Benke 1994; Grubaugh et al. 1997). The four groups of approaches were reviewed by addressing the following five key questions. (1) What was the method designed to do? (2) What are the assumptions and limitations? (3) What measurements are required? (4) How has the method been used in a habitat context? (5) Does the method produce an estimate of PC, or an acceptable surrogate thereof, or does it require additional data and calculations to complete the quantification? Much of the information was summarized in tables but discussion of key aspects, particularly assumptions and limitations, was provided in the text. This review of approaches focuses on components 4–6 of the assessment framework (Table 1).

Given the prior definitions of PC and the framework outlined, the four groups of approaches represent an upward pro-

Table 1. Components of an assessment of no net loss of productive capacity (PC) of fish habitats.

Component	Description	Commentary
1. Frames of reference (fish and ecosystem)	Identification of the ecosystem, the habitats, and the fishes that will be assessed.	All native species present, or that might be expected to be present, in the ecosystem are to be the default reference point for an assessment. Non-native species can be included as appropriate.
2. Assignment of weights	Relative weights are assigned to all fish and life stages identified. The relative weights represent efforts by management agencies to balance among social, economic, and ecological factors. Specification of weights is a management responsibility.	Socioeconomic factors may include fishery preferences, species-at-risk, or exotic or invasive species, etc. Ecological factors may include rarity, relative contribution to productivity or biomass, trophic level, dietary contribution in prey, etc. For example, weights might be used to distinguish between native and non-native species, with the former being given higher weights.
3. Habitat mapping	Designation of identifiable habitat types and quantitative assessment of their extent by habitat type. Area is the usual basis for spatial assessment, although length or volume might be relevant in some instances.	Habitat types should be specified with regard to the requirements of all fishes, though schemes that lead to very large numbers of types can be difficult to assess.
4. PC metrics	PC metrics for habitat are specified on a relative scale (usually 0 to 1) for all fish elements with respect to all habitat types.	Suitabilities are the most commonly used metrics. The specification of suitability may be based on assessments of selection for, preference for, relative performance or fitness, or similar for fish elements.
5. PC units	The absolute PC units provide a means of scaling from relative productivities expressed in suitability values to some absolute measure of productivity (mass per area per unit time).	Production rate per unit area is the ideal choice but often cannot be estimated when some required PC metrics (growth rate, CPUE, presence or absence) are not available.
6. Computing PC supply	The sum over all fish elements and habitat types of the product of spatial extents (component 3) and PC metrics (component 4) and possibly with the absolute scalar (5) leads to a total PC supply with the weights applied (component 2).	Within an ecosystem, relative metrics (see component 4) will suffice. Absolute units (see component 5) may be needed to balance the consideration of all issues in a societal context. However, it should be noted that socioeconomic valuations of productivity must be based on the cost of developing and sustaining habitats that can supply the production and not the mere cost of stocking equivalent amounts of fish.
7. Net change assessments	Explicit comparison among alternate habitat configurations, one of which is a predevelopment baseline or reference scenario. Differences are estimates of net change.	Various scenarios can be examined as part of a design process leading to a preferred option that prevents a net loss or minimizes losses when unavoidable.
8. Uncertainty, risk, and timing	Explicit consideration of methodological incompleteness and the uncertainties and timing issues surrounding assessment of net change	Methodological completeness is balanced with the precautionary principle. Uncertainties (measurement error, spatial and temporal variability, specification errors, etc.) can affect the potential for discerning net change or the differences among alternate options (Minns 2006). Risks of success or failure vary. Timing issues such as when losses precede gains in a development affect net change, as does the temporal discount rate (Minns and Moore 2003)
9. Habitat cofactors	Habitat factors that were not captured in the basic mapping of physical habitat features.	Cofactors might include the hydrological and thermal regimes, climate variability and change, or levels of beneficial (nutrients) and detrimental (toxicants or contaminants) chemical attributes of habitat features. The spatial configuration and connectivity of habitat patches also needs to be considered.
10. Biotic cofactors	The lists of fish elements that make up the focus of an assessment may not fully take account of thresholds, interactions, and feedbacks operating among biota in the ecosystems.	Factors such as depensatory population growth thresholds, predator–prey or host–parasite interactions, indirect effects of fisheries harvests, etc., may shape responses to habitat changes beyond the immediate habitat impacts. Invasives species may constrain productivity

gression in which the level of attainment of the measurement of PC (question 5 above) depends on completeness and the extent of calibration and validation. The habitat- and individual-based methods can only represent components 4–6 in a relative manner, and then their use may only be based on broad inference unless specific calibration evidence is presented. The population- and community–ecosystem-based methods may also provide only a relative scaling of PC unless full production calculations are involved.

Habitat-based methods

Included in this group are methods that exclusively measure physical habitat properties of ecosystems and, less frequently, those that directly measure physical habitat features

but then make assumptions or inferences about the biological use of those features to make quantitative assessments of the habitat change. Hence, these methods do not actually measure PC, although they are widely used as surrogates to guide fish habitat managers. Habitat-based methods can be classified into four subcategories: (*i*) hydrological methods; (*ii*) hydraulic rating curves; (*iii*) habitat simulation models; and (*iv*) defensible methods. Each category addresses different purposes, requires specific data, makes certain assumptions, and comes with its own limitations (Table 2).

Historically, all of these habitat-based methods have been used to some degree to assess impacts of human development on fish habitat in Canada. Measuring physical properties of systems is often easier and less expensive than quantifying attributes of fishes or a biological surrogate, particularly if the ultimate goal is to measure fish production. Hydrological and hydraulic rating methods are most frequently applied to calculate minimum flows (instream flow needs) for fluvial environments in the context of water abstractions or establishing minimum flows below dams or diversions. They are suitable for application when risk to fish habitat is considered low and a quick, but quantitative, desktop or minimal data requirement method is desired. Although hydrological (40%) and hydraulic rating (5%) methods collectively comprise 45% of environmental flow methods used in Canada, habitat simulation is also extensively used, making up an additional 45% of methodologies (Tharme 2003). Strictly speaking, hydrological and hydraulic rating methods are not suitable for assessing NNL as they involve no measure of biological change and, therefore, technically do not represent PC.

Habitat simulation models predict change in habitat availability resulting from incremental changes in flow, which are then linked to a composite habitat suitability index (HSI), based on habitat preferences for fish species of interest, to ultimately arrive at weighted useable areas (Ahmadi-Nedushan et al. 2006; de Kerckhove et al. 2008). Although habitat suitability models could be used to calculate NNL, their data-intensive nature means that they are more often applied in situations in which multistakeholder interests are considered and objectives are broader than simply achieving NNL (e.g., Katopodis 2003). Criticisms of HSI models focus on assumptions, specifically the assumed linear relationship between habitat area and population biomass, and that HSI curves are independent of flow conditions, both of which have been demonstrated to be false (Holm et al. 2001; Vilizzi et al. 2004). HSI transferability between locations has been questioned (Guay et al. 2003; Nykänen and Huusko 2004) but has also been shown to be regionally supported (e.g., Mäki-Petäys et al. 2002). Transferability is not an issue when HSIs are developed for the fishes in the system in question via direct measurement. Regardless, habitat simulation models continue to be used and improved for use in environmental flow assessments. Attempts at improvement include increasing the scale of habitat measurements to make them more relevant to management decisions (i.e., MesoHABitat SIMulation or MesoHABSIM; Parasiewicz 2001, 2007*a*, 2007*b*) and integrating dynamic ecological feedbacks (such as competition and food supply) into models (e.g., Anderson et al. 2006).

Defensible methods (Minns and Nairn 1999) is the only method in this category that was specifically created to provide a scientifically robust approach to the application of DFO's Policy for the Management of Fish Habitat (DFO 1986) and that is suitable for application to lacustrine environments. This approach assumes that there is a direct relationship between amounts of suitable habitat and PC. The conceptual approach of defensible methods is similar to habitat simulation models in that it assigns relative suitabilities to habitat and calculates useable areas as a basis for assessing change, but it is significantly different in that all fish species and life stages affected are taken into account and only parts of lake ecosystems can be assessed in isolation of the whole (Minns and Nairn 1999). The initial implementation of the approach was designed for application to Great Lakes nearshore habitat. To facilitate its use by the HMP, a literature-based database of fish species by life stage habitat requirements was compiled (Lane et al. 1996*a*, 1996*b*, 1996*c*), and computer software was developed. Validation of field predictions is an important next step to increase confidence in the approach (Minns and Nairn 1999). Nonetheless, the correct application of defensible methods allows for the calculation of compensation habitat required to achieve NNL and for objective discussions with the proponent around a quantitative and consistent assessment of the impact of the proposed development. When the scale of the project warranted its application (e.g., Diavik diamond mine in the Northwest Territories), defensible methods has been successfully used by the HMP and, had it been applied more frequently, would have resulted in better achievement of NNL than traditional, less quantitative file reviews (MacNeil et al. 2008). Current efforts are underway to develop an application suitable for southern Ontario streams (realignments, crossing) and to compile a report documenting all the features of the software package that go beyond its current practical use and the regional habitat requirements databases available for regional application of the tool.

Individual metrics and vital rates

Individual fish measurements of habitat quality, including body size, individual growth, condition, fitness, health, and behaviour (Minns et al. 1996*a*; Pearson et al. 2005) (Table 3), provide a key part of the information needed to calculate PC and might be used as surrogate indicators. Fitness combines two metrics as it is a function of both growth potential and mortality risk (Rosenfeld and Boss 2001; Railsback et al. 2003; Rosenfeld and Taylor 2009). The vital rates of population growth, survival, and reproduction are also discussed in this section as individual measures of habitat quality, to provide a transition from individual to population approaches, and also to emphasize the constraints and limitations of interpreting individual measures by themselves as scalars of habitat quality and quantity. Constraints and limitations are discussed in the context of (*i*) interdependence and interaction among measures, (*ii*) methods, and (*iii*) future application as defensible measures of habitat quality.

Individual metrics are sometimes overlooked as measures of PC because managers often focus on population or community metrics (Pearson et al. 2005). Individual metrics were included in a review of methods for measuring PC in Canada, but usually as subcomponents of habitat- or population-based models (Smokorowski and Derbowka 2008). Metrics of fish health (parasites, tumours, disease, morphology, physiology)

Table 2. Habitat-based methods of assessing productive capacity (PC).

Method (examples)	Purpose	Data Needs	Assumptions and (or) limitations	References
Hydrological method (e.g., Tennant, median monthly flow — Q50, 7Q10)	• Based on flow data only • Designed to be a simple desktop exercise without the need for rigorous field data collections	• Uses some percentage of historical flows (or predictions of MAF based on catchment characteristics) or rules from flow duration curves	• Relies on assumptions between flow and habitat and usually is not calibrated to the watershed in question	Tennant 1976; Caissie and El-Jabi 1995
Hydraulic rating curves (e.g., wetted perimeter, transect method)	• Correlation between hydraulic parameters and physical habitat features at specific transects across a river	• Vary but can include wetted perimeter, water surface width, velocity, depth, cross-sectional area, and substrate size	• Direct measurement of systems avoids many assumptions • Simple data to collect but lacks the link to biological attributes and results in a single recommended flow value	O'Shea 1995; Reinfelds et al. 2004; Kilgour et al. 2005
Habitat simulation models (e.g., IFIM, PHABSIM, River 2D, MesoHABSIM)	• Specific habitat features are the focus (e.g., depth, substrate, velocity, cover) and are simulated at different flows to give WUA for species of interest	• Similar to hydraulic rating but at more transects to cover a broader area of river • MesoHABSIM uses larger scale habitat features (riffle, run pool, etc.) under multiple flow conditions • All require a component of biological knowledge of habitat use by animals preferably calibrated to the watershed in question	• Biggest criticism is the weakness of habitat suitability curves relating standing crop to habitat features and the transferability of habitat suitability curves across systems	Stalnaker et al. 1995; Parasiewicz 2001, 2007*a*, 2007*b*; Katopodis 2003; Parasiewicz and Walker 2007
Defensible methods (e.g., HAAT)	• To develop an equation that can calculate the net change in PC of habitat affected by human developments	• Area affected by development (pre- and post-development, including any compensation habitat) • PC of the habitat before and after development • Potential maximum PC of the lost habitat	• Estimates of habitat value often dependent on expert panels or habitat surrogates • Assumes a fixed value of habitat for each species and life stage and does not take adaptability into account • Requires a large amount of information to validate	Minns 1997; Minns and Nairn 1999; Minns et al. 2001

Table 3. Individual-based metrics for estimating or inferring productive capacity.

Metric	Purpose	Data needs	Assumptions and (or) limitations	References
Body size	• Use of fish body size as an indicator of habitat type	• Habitat-dependent body size	• Link between body size and population vital rates	Schlosser 1990; Harvey and Stewart 1991; Crozier et al. 2010
Condition	• Use of condition factor as an indicator of habitat quality	• Estimates of condition and accepted reference	• Usually used as a measure of fish nutrition status • Assumes link between condition and population vital rates • Highly variability	Copeland et al. 2008; also see references for the health metric
Health	• Use of fish health as an indicator of environmental quality	• Indicators and quantification of fish health	• Used as a tool for measuring water or environmental quality rather than habitat quality	Munkittrick and Dixon 1989; Adams et al. 1993
Behaviour or movement	• Use of localized fish movement as an indicator of habitat quality	• Movement observations by habitat type also should have individual growth and (or) survival observations	• Assumes movement benefits vital rates • Tools and methods are often species- or habitat-dependent and expensive	Bélanger and Rodríquez 2002; McMahon and Matter 2006; Dolinsek et al. 2007
Growth	• Use of fish growth rate as an indicator of habitat quality	• Direct growth observations by habitat type and (or) bioenergetic model	• Assumes direct link between habitat quality and growth, but interacting factors may be confounding	Jones and Tonn 2004; Gilliers et al. 2006; Amara et al. 2009; Rosenfeld and Taylor 2009; Crozier et al. 2010
Survival	• Use of habitat-dependent survival rate as an indicator of habitat quality	• Direct survival estimates by habitat type	• Must distinguish between emigration and mortality	Gorman et al. 2009; Laurel et al. 2003
Fitness	• See survival and growth purposes	• Growth and survival estimates by habitat type	• Statistical method to combine the above estimates to measure fitness	Railsback et al. 2003; Rosenfeld 2003; Harvey et al. 2005
Reproduction	• Recruitment success as an index of habitat quality	• Direct recruitment estimates by habitat type	• Recruitment is a limiting factor to population production • Long-term and spatially extensive data are needed	Knapp et al. 1998; Baglinière et al. 2005; Johnson 2007

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and condition factor, in conjunction with population indicators, have been used to assess water quality and other environmental factors or stressors (e.g., mine waste, pulp mill effluents, reservoir management) (e.g., Munkittrick and Dixon 1989; Goede and Barton 1990; Adams et al. 1993) rather than to assess habitat quality per se. Fish body size has important fitness consequences (Crozier et al. 2010) and has been related to habitats at both landscape (Schlosser 1990) and site scales (Harvey and Stewart 1991; Heggenes et al. 2002) but is most effective as a measure of habitat condition if used together with other individual and population indicators (Crozier et al. 2010). Pearson et al. (2005) recommended the use of individual-based biotic variables in habitat assessments for two reasons: (*i*) differences in body size, condition, and behaviour are very sensitive indicators of environmental change and provide insight into mechanistic links between habitat and fishes; and (*ii*) individual metrics have high statistical power compared with population measures (Osenberg et al. 1994).

Directed fish movements are a behavioural response of individual fish to gradients in habitat quality to seek cover (survival), efficient foraging (growth), or successful reproduction (Lomnicki 1988; Sutherland 1996; McMahon and Matter 2006). Because of these functional links to habitat, localized movements have been proposed as a preferred measure of habitat quality over traditional density–quality relationships for stream salmonids (Bélanger and Rodríquez 2002). Hayes et al. (2009, p. 296) using five case studies from the Great Lakes concluded that there is a "paucity of field-based data directly relating birth, death, and movement rates to habitat conditions experienced by individual fish". Historically, studies of fish movement in the field were time consuming and species- and habitat-dependent, limiting their application as a tool for habitat management, but recent technological advances (e.g., telemetry, video, acoustics) have increased the range of techniques available, allowing biologists to better track spatial behaviour and to determine habitat preference more broadly (Lucas and Baras 2000; Spedicato et al. 2005).

Growth, survival, and recruitment are the key components of population production, and each of these vital rates varies with habitat heterogeneity and quality. Examples of research on habitat-dependent vital rates include growth (Jones and Tonn 2004; Rosenfeld and Taylor 2009; Crozier et al. 2010), survival (Laurel et al. 2003; Gorman et al. 2009), fitness (Railsback et al. 2003; Rosenfeld 2003), and reproductive success (Knapp et al. 1998; Pihl et al. 2005; Johnson 2007) (Table 3). A common constraint with using these metrics individually in a PC context is that they are not independent. To be defensible indices of habitat quality, it is necessary to investigate the vital rates collectively, to better understand interactions, and to link the individual metrics to net effects on populations (Railsback et al. 2003; Harvey et al. 2005; McMahon and Matter 2006). Vital rates are best explored collectively using process-oriented habitat–population models (Hayes et al. 2009), as discussed under the model section.

Individual metrics are included, explicitly or implicitly, in PoE diagrams (noted earlier) that link in-water activities to habitat change and subsequently to population effects. An example is potential impacts to movements, recruitment, and growth of migratory trout caused by stream crossings (Jones et al. 1996). The PoE diagrams are used by habitat managers and proponents to assist in identifying mitigation actions needed to avoid the negative impacts to individual metrics. Validation of the flow management pathway included the science review of many of the individual metrics discussed in this section (Table 3), as well as the interactions among metrics (Clarke et al. 2008).

For the PoE process, the default but reasonable assumption is that potential negative effects on individual metrics are undesirable. Limitations and uncertainty associated with using individual metrics for measuring PC could be overcome by developing peer-reviewed guidelines and by undertaking meta-analysis of the science literature (e.g., see Smokorowski and Pratt (2007) and Jensen et al. (2009)). Synthesis of information on relationships between individual metrics and fish habitat to obtain general science-based rules can be used as surrogates for component 4 (assigning suitabilities) within the NNL assessment framework (Table 1) as done using growth potential by Jones et al. (2003).

Population-based approaches

Population-based methods traditionally have been used to measure fish responses to habitat changes. Population-based methods vary from simple to complex: relative or absolute abundance (catch per unit effort, density, biomass); population structure; production rate; stock–recruitment relationships; and more recently, habitat–population models (Table 4). The extent to which population approaches fulfil the conditions laid out for complete NNL assessments (Table 1) depends on whether abundance measures like catch-per-unit-effect are used alone or in combination with vital rates to estimate production rates. Abundance methods mainly represent partial or surrogate indicators, whereas rate-adjusted methods are likely to be more complete, although production calculations may not be an explicit output of the method.

Historically, in Canada and elsewhere, population responses to habitat manipulations were often not assessed (Smokorowski et al. 1998; Quigley and Harper 2006). If assessments were done, the frequency of use of the different methods decreased with complexity: abundance (biomass) was measured often (Quigley and Harper 2006), and production rarely (Smokorowski et al. 1998). Population–habitat models were introduced relatively recently, and their use is increasing, particularly for quantifying habitat needs for recovery of species at risk (e.g., DFO 2007*b*). This brief critique of the application of population methods in Canada (i.e., assessing NNL) indicates that the assumptions (and uncertainty) decrease, but the data and knowledge needs increase with method complexity.

For quantifying habitat quality for species at risk, if ecological gradients of habitat use and habitat features are known through surveys, generalized linear models (GLMs) or generalized additive models (GAMs) can be used to fit data on species abundance to habitat features (DFO 2007*b*). GLMs assume the habitat–abundance relationships are linear (or are transformable to linear) over a range of habitat features and are statistically slightly more powerful than GAMs. Detecting discernible ecological gradients of habitat use from empirical data assumes that there are gradients in PC.

Measuring response of fish abundance to habitat alteration to assess NNL assumes a direct link between abundance and habitat quality (fitness), an assumption that is often not tested

Table 4. Population-based assessment methods and models for measuring productive capacity.

Methods	Purpose	Data needs	Assumptions and (or) limitations	References
Abundance (CPUE, density, biomass); GLMs; GAMs	• Traditional measure of population response	• Relative (CPUE) or absolute abundance (density) by habitat category	• Link between abundance and population production is inferred • Abundance is habitat-limited • Abundance is related to habitat quality and function: food supply, refugia, reproduction, movement • High variance in abundance data limits statistical precision	Smokorowski et al. 1998; Minns et al. 1996*a*; Clarke and Scruton 2002; Quigley and Harper 2006; DFO 2007*b*
Population structure (age, body size, growth, production/biomass (*P*/*B*))	• Biological indices or surrogates of production as an intermediate measure between abundance and production	• Seasonal mean biomass and *P*/*B* • Body size, age structure, and seasonal growth	• Site data reflect population structure • Population structure is habitat-dependent • Production can be compartmentized by habitat type • Static data are used to make inferences regarding dynamic process	Minns et al. 1996*b*; Randall and Minns 2000; Jones et al. 2003; Pratt 2004; Cote 2007
Production (g m^{-2}·$year^{-1}$)	• Fish production is the measure of habitat capacity implied by habitat policy • Measure link between habitat and productivity	• Recruitment, seasonal abundance and survival, biomass, and growth by life stage	• Survey areas are representative of habitat and ecosystem • Requires large field effort • Used for stock assessment but rarely for habitat assessment	Hunt 1974; Smokorowski et al. 1998
Stock–recruitment (*S*–*R*) models, including stage-structured models	• Identify population carrying capacity • Evaluation of habitat effects on population status	• Long-term data sets (>10 years) • Spawning stock size and recruitment abundance • Habitat-associated survival	• Recruitment is determined by numbers of spawners and habitat supply • Requires long-term monitoring of fish response to habitat restoration to validate models	Sharma and Hilborn 2001; Gibson 2006; Lobón-Cerviá 2007; Mounalli and Hilborn 1986; Scheuerell et al. 2006; Honea et al. 2009
Stage-structured, habitat supply models	• Quantify habitat needs for recovery of species and habitat management	• Quantitative links between habitat and vital rates for life stages	• Do not deal with habitat quantity and quality simultaneously	Minns et al. 1996*b*, 1999*a*; Minns 2003; Vélez-Espino and Koops 2009*a*
Statistical models	• Link population characteristics to multiple habitat features	• Statistical links are established using complex techniques such as MLR and ANN	• Often difficult to determine which inputs are most influential	Fausch et al. 1988; Laë et al. 1999; Lek et al. 1996
Individual-based models	• Model population response to habitat conditions	• Vital rates linked to environmental conditions, fish transport, and movement behaviour	• Paucity of field-based data relating birth, death, and movement rates to habitat conditions at fine temporal and spatial scales • Large data needs	Tyler and Rose 1994; Rose 2000; Hayes et al. 2009

Note: Data needs and complexity increase from top to bottom.

or is shown to be invalid if tested (Van Horne 1983; Rose 2000; Railsback et al. 2003). In their review, for example, Clarke and Scruton (2002) cited studies showing an increase in salmonid density or biomass in habitats in which compensation efforts were applied to offset losses elsewhere but were uncertain if this reflected increased production or fish redistribution. Uncertainty of a direct link between fish abundance and habitat quality is a major weakness. Aside from precision issues (high variance; Minns et al. 1996*a*; Quigley and Harper 2006), there are several reasons why fish abundance and habitat quality may not be correlated (Rose 2000; Railsback et al. 2003). Three factors are mentioned here to demonstrate that the confounding factors are both directly or indirectly related to habitat: (*i*) nonlinear and nonproportional responses to complex spatial heterogeneity in habitat (Rose 2000; DFO 2004); (*ii*) population responses are affected by fish community interactions (Rose 2000; Railsback et al. 2003); and (*iii*) cumulative effects of multiple stressors, both habitat and nonhabitat related, can mask direct links between habitat quality and fish abundance (Rose 2000; Harvey and Railsback 2007).

Measuring abundance responses to habitat alteration requires knowledge of the production mechanisms that determine the key fitness elements of habitat — reproduction, growth, and survival. For an artificial stream in the Canadian Arctic, PC was assessed by comparing growth, biomass, and movements of Arctic grayling (*Thymallus arcticus*) at an artificial and reference stream (Jones et al. 2003). For example, adult grayling successfully moved through the artificial channel, spawned, and produced fry, but growth rate and biomass of fry in the artificial stream were lower than those found in the reference stream because nursery habitat was deficient (reduced habitat complexity, food supply, temperature). This mechanistic approach to assessment revealed both positive and negative population effects and was instructive for planning and advancing future compensation measures.

More complex population methods utilize size structure information of fishes to make inferences about production to biomass ratios (*P*/*B*) and productivity (Randall and Minns 2002; Pratt 2004; Cote 2007). Assumptions related to abundance apply to these biological indices of production as well. Because of the cost and data requirements, estimates of production rate continue to be used more often for fisheries than habitat management.

Stock–recruitment (*S–R*) relationships quantify area-based survival, and with sufficient time series of data, population productivity can be estimated, providing information to habitat managers on regional and site-specific factors affecting carrying capacity (Sharma and Hilborn 2001; see Gibson (2006) and Parken et al. (2006) for Canadian examples). *S–R* data usually involve large spatial-scale analysis (ecosystems). A multistage *S–R* model (Moussalli and Hilborn 1986) was used to measure habitat effects on the status of *Oncorhynchus* spp. (Sharma and Hilborn 2001; Scheuerell et al. 2006; Honea et al. 2009) by explicitly linking survivorship between different life stages to location and specific habitat parameters (e.g., fine sediment, temperature, habitat structure, pools, gradient). Using a different approach, Lobón-Cerviá (2007) used *S–R* data at a site rather than a river scale to show the importance of site depth and discharge on the survivorship of brown trout (*Salmo trutta*). Significantly, *S–R* models traditionally used for fisheries assessment at an ecosystem scale are now being used to quantify habitat–population linkages at regional and site scales. *S–R* data and production estimates have rarely been used for habitat management in Canada, but their use will likely increase in the future (Randall 2003).

Population-based methods for assessing PC are useful in ecosystems dominated by one or a few species (e.g., salmonids), but community-based approaches are more often used in species-rich areas of Canada such as the Laurentian Great Lakes basin (Randall and Minns 2002). More sophisticated habitat–population models, recently used for the management of endangered species, are discussed in the section on habitat–population and ecosystem models.

Habitat–population models linking population traits to habitat

The strength of mechanistic habitat–population models is that they consider causal relationships between changes in habitat and gain or loss in fish production (de Kerckhove et al. 2008). Two categories of models merge habitat-explicit information and population dynamics at different levels of information: (*i*) unstructured population models focusing on a specific life stage or aggregating individuals from all stages regardless of their habitat preferences; and (*ii*) structured population models explicitly dividing the life cycle into life stages (Table 4).

Examples of the first category are the habitat suitability index and *S–R* models, both described briefly in previous sections. Also included are multiple linear regression (MLR) models (Fausch et al. 1988) and artificial neural networks (ANN) (Laë et al. 1999) used to predict fish production (Table 4). The advantage of ANN over MLR models is the ability of ANN to assess nonlinear relationships. Multiple linear regression and ANN have been combined to develop stochastic models of fish production using habitat features on a microhabitat scale (Lek et al. 1996).

The second category of models requires accommodating ontogenetic shifts in vital rates (e.g., growth, survival, fecundity) and habitat requirements. Spatial distribution and connectivity among habitats used by different life stages can represent important modelling features (Schindler and Scheuerell 2002). Two modelling approaches fall within this category (Table 4): (*i*) stage-structured matrix models that consider all individuals within a stage identical; and (*ii*) individual-based models that explicitly incorporate individual differences in behaviour and response to habitat conditions (e.g., Humston et al. 2004). Some advantages attributed to individual-based models such as the ability to explicitly incorporate spatial distribution and heterogeneity can be achieved at the population level with multisite matrix models (Caswell 2001; Morris and Doak 2002) through the incorporation of spatially explicit dynamics and (or) vital rate variation within life stages (Vélez-Espino and Koops 2009*a*).

Structured habitat–population models have been used to develop management strategies for recovery of endangered species. The most common limitation of structured population models is that they focus on a particular vital rate or life stage (Brandt and Kirsch 1993; Mason et al. 1995) and cope with changes in either habitat quality or quantity. Merging the combined effects of changes in quantity and quality of habitat on PC should be considered. Habitat–population mod-

els require both habitat preferences of all life stages and functional relationships between habitat variables and vital rates (Cowan et al. 2000; Hayes et al. 2009). Important improvements could be achieved by focusing on those functional relationships with the greatest contributions to population performance (Vélez-Espino and Koops 2009*b*). It is important to distinguish between evaluations of "essential fish habitat", based on the analysis of population responses to changes in vital rates, assuming no habitat constraints (e.g., Levin and Stunz 2005), and "limiting habitat," which is the stage-specific habitat most sensitive to management interventions and the demographic sensitivities of the vital rates of life stages using that habitat (Vélez-Espino and Koops 2009*b*).

Habitat supply models (Minns et al. 1996*b*, 1999*a*; Minns 2003), which are based on the assumption that stage-specific survival decreases in proportion to the ratio between habitat supply and habitat requirements below a threshold, identified as the critical area, have been used to measure the effect of habitat loss on population growth (Vélez-Espino and Koops 2009*b*). Although individual-based models appear to be the best approach to model population responses to habitat conditions (Hayes et al. 2009), the paucity of information at fine scales for important habitat variables (Hayes et al. 2009) favours the use of methods explicitly separating life stages but demanding less information. Nonetheless, any type of habitat–population model still requires a known or assumed functional relationship between some habitat component (biotic or abiotic) and population attributes (e.g., Hayes et al. 1996; Rodwell et al. 2003; Eby et al. 2005).

Future application of habitat–population models to estimate no net loss

Balancing the efficacy with the feasibility of measuring PC may require a combination of approaches to guide habitat management under the NNL principle. Given the trade-off between the level of assessment of PC, quality of data, and number of assumptions of habitat–population models, it may be appropriate to follow a holistic approach in which insights gained from an aggregated model encompassing the entire community or fish assemblage (discussed in next section) can be corroborated or complemented with a structured model (category 2 above) of an indicator species. Indicators can be represented by strongly interactive species, which at low abundances can trigger ecological cascades of degradation and species loss (Soulé et al. 2005), which would also be highly relevant from a diversity standpoint. For those cases with access to rich biological and ecological information, including knowledge of spatial and temporal variation, an individual-based model should be favoured. Otherwise, a single-site or multisite matrix model could be used to assess PC at the population level.

For habitat–population models, it is a challenge to determine the true nature of the relationships (linear or nonlinear) between habitat quantity or quality and population vital and movement rates, which could strongly influence predictions of PC (e.g., Rodwell et al. 2003). In addition, because area–abundance curves change with habitat carrying capacity, the integration of habitat quality and quantity in a single analysis must address density-dependent processes (e.g., Englert et al. 2000). One effective way of incorporating habitat suitability information into density-dependent models is through the use of software programs (e.g., RAMAS GIS/Metapop, RAMAS Ecological Software, Setauket, New York) linking map overlays with metapopulation dynamics (e.g., Root 1998). Moreover, cumulative effects of multiple stressors of habitat quality could be incorporated for projections of PC. This is important because synergistic effects can be missed by short-term studies focusing on individual habitat variables (Rose 2000) and because aquatic habitats are subject to cumulative impacts from diffuse activities (Schlosser 1991). Finally, source–sink dynamics and connectivity should be understood and assessed to guide the placement and creation of new habitat or the protection of specific areas (Crowder et al. 2000) within the framework of NNL.

Community- and ecosystem-based approaches

By definition, PC is a habitat characteristic that is measured, directly or by surrogate, as fish production. Early attempts to provide an operational definition emphasized that the concept of PC implicitly included biodiversity (Jones et al. 1996). Fish community changes resulting from habitat alteration are likely a more sensitive indicator (Minns et al. 1996*a*) than changes in fish biomass and production. Minns et al. (1996*a*) listed five fish community measures that have been used to detect response to habitat changes: species richness; community biomass and productivity; composition (species, size, trophic); and interspecies distribution. Multimetric measures have also been used (Table 5). As with population approaches, the degree of compliance with the assessment requirements (Table 1) depends on the extent to which vital rates and abundance measures are used to estimate production rates. Nonproduction methods will provide surrogate indicators. Community- and ecosystem-based methods provide a greater potential to account for overall changes in PC.

For assessing the effectiveness of habitat compensation, Quigley and Harper (2006) measured both fish diversity and biomass as proxies of PC. Quigley and Harper (2006) and others (Minns et al. 1996*a*; Rose 2000) have emphasized that because of inherent ecosystem variability, differences in community measures between sites must be large to detect responses. Another constraint is that the efficacy of fishing gear is often habitat-dependent (Portt et al. 2006), and multiple gear types are needed to measure fish diversity in different habitats (Jackson and Harvey 1997).

To effectively measure PC, community-based approaches often go beyond measures of diversity. Minns et al. (1994) developed an index of biotic integrity (IBI) for littoral habitats, which utilizes data on fish trophic composition and abundance, as well as species diversity. IBI was originally developed and successfully applied to rivers (Karr 1991; Wang et al. 1997), and fish metrics have proven useful for measuring perturbations in other habitats such as estuaries (Deegan et al. 1997; Meng et al. 2002). Metrics for application of the IBI in both lakes and rivers are sometimes redundant (Minns et al. 1994; de Kerckhove et al. 2008) and may need site-specific modifications (Long and Walker 2005).

Randall and Minns (2000) developed a habitat productivity index (HPI), calculated as the sum of the average seasonal biomass times *P*/*B* for all species in a particular habitat. The HPI assumes that community production is positively correlated with community biomass and that community biomass

Table 5. Community-based assessment methods and models for measuring productive capacity (PC).

Methods	Purpose	Data needs	Assumptions and (or) limitations	References
Biodiversity	• Measure habitat conservation	• Species richness and relative abundance	• Inherent ecosystem and habitat variability in community diversity • Multigear types needed for different habitats	Quigley and Harper 2006
IBI	• Measures ecosystem health based on four factors: habitat supply, water quality, exotic species, and piscivore abundance	• Fish assemblage measures of species richness and trophic composition	• Selection of metrics may be subjective • Not as useful in species-poor areas	Minns et al. 1994 Long and Walker 2005
Habitat productivity index (HPI)	• Designed as a field measure of PC	• Seasonal biomass and production/biomass (*P/B*), all species	• Assumes biomass summed for all species measures PC • Difficult to obtain quantitative data in all habitats • Factors other than habitat (e.g., exploitation) may affect biomass	Randall and Minns 2000, 2002
Multimetric ecosystem approach	• Measures ecosystem productivity	• Fishes, invertebrates, habitat	• High variability	Roth et al. 2007 Jones et al. 2008 Gavaris 2009
Fish assemblage models	• Usually used to assess marine protected areas	• Fish biomass (community) in protected area and at fishing grounds • Habitat quality in protected area • Habitat quality – mortality relationship	• Assumed habitat quality – mortality relationship • Uncertainty of habitat and density-dependent fish movement	Rodwell et al. 2003
MEI	• Estimate fish yield	• Lake morphology, TDS	• Limited to lentic systems	Ryder 1982
Mass–balance (EcoSpace)	• Compare management scenarios	• Biomass of functional groups, *P/B*, diet, spatial dynamics	• Uncertainty of seasonal changes and directed migration • Large data needs?	Walters et al. 1999
Multiple trophic level models	• Integrated production	• Biomass, *P/B*, diet	• Applied to coastal wetlands	French McCay and Rowe 2003

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is a function (and thus a measure) of habitat productivity. Both assumptions require further research for validation. For species-rich nearshore areas of the Great Lakes, Randall and Minns (2002) recommended a two-axis approach, using IBI and HPI simultaneously to measure the diversity and productivity components of PC, respectively. In addition to fish measurements, Quigley and Harper (2006) measured periphyton and invertebrate biomass and the density of riparian vegetation, using a multimetric ecosystem approach, for measuring PC. Jones et al. (2008) also used a multitrophic approach to measure compensation habitat in Canada's arctic. Roth et al. (2007) assessed the role of woody habitat, impacted by residential development, on fishes and food supply. Although effective, these multimetric approaches have rarely been used in Canada because of their high cost.

Multimetric, community approaches for measuring habitat capacity are consistent with the recent focus on the ecosystem in conservation ecology, both in Canada and internationally. Together with maintaining productivity and preserving biodiversity, protecting the habitat needed to sustain aquatic resources is one of the three key conservation principles used to manage in-water and land-based human activities that impact aquatic resources (Gavaris 2009). Although challenging, managing fish habitat as an ecosystem component is a critical priority in both freshwater and marine areas.

Community and ecosystem models

Ecosystem science has become the foundation for the integrated management of diverse human activities that are regularly undertaken in a common area (DFO 2007*a*). The use of ecosystem-based management (EBM) in Canada (e.g., Gavaris 2009) is consistent with global approaches (e.g., International Council for the Exploration of the Sea, European Commission) for managing habitat, fisheries, and endangered species. Ecosystem models used to assess changes in the PC of habitat have ranged from simple applications of the morphoedaphic index (Ryder 1982), through aggregate community models, to complex computer simulations of trophic flows among the functional groups of an ecosystem (e.g., Ecopath: www.ecopath.org) (Table 5).

The morphoedaphic index (MEI, total dissolved solids ($mg{\cdot}L^{-1}$) divided by mean depth (in metres)) has been used globally as a pragmatic estimator of fishing yield on the basis of constraints in productivity being determined by lake-basin morphometry and nutrient and energy availability (Ryder 1982; Jenkins and Oglesby1982). The MEI applies only to lentic systems and, in principle, can be applied to any set of freshwater (Ryder 1982) or saltwater (Khalil 1997) lakes or reservoirs within a region with relatively homogeneous climatic conditions. However, it has been demonstrated that MEI is more effective in natural lakes than in reservoirs given greater index variability in reservoirs during the first decades of impoundment (Jenkins and Oglesby 1982). Species interactions affect the efficiency of the MEI to assess productivity changes at the population level (Hubert and Guenther 1992), and therefore, MEI seems more appropriate as a productivity surrogate at the community level. Also, there are statistical problems with MEI as it employs ratios (Jackson et al. 1990). Other limnological indices used to assess aquatic productivity in lakes include shoreline development, primary production, and nutrient input (Downing et al. 1990; Khalil 1997; Hayes et al. 2009).

Aggregate community models usually rely on difference and steady-state equations and have been used to determine the importance of habitat quality and quantity for protected areas and marine reserves (Mangel 2000; Rodwell et al. 2003). As the definition of PC is the sum of the production rates for all cohabiting fish species within a defined area (Minns 1997), these aggregated models are relevant for measuring PC but were originally designed to explore different management scenarios (e.g., Rodwell et al. 2003). Data requirements of these aggregate models are difficult to meet. Assumed parameters, or borrowing data from other species, may lead to substantial model uncertainty (Hayes et al. 2009).

The use of dynamic simulation models of biomass fluxes' responses to ecosystem modifications (e.g., fishing, eutrophication) as tools to evaluate management scenarios has increased since the development of the model suite ECOPATH, ECOSIM, and ECOSPACE (Walters et al. 1999; Pitcher et al. 2002). ECOPATH is a mass-balanced model of an ecosystem's trophic flows that can be used to structure dynamic simulations using ECOSIM. Ecosystem attributes such as dispersal rates, foraging, and predator avoidance can be incorporated for a defined range of habitats through the use of ECOSPACE, which has been used to evaluate the interactions between policy scenarios, catch returns, and shifts in species compositions in marine protected areas (Pitcher et al. 2002). Although ecosystem projections using ECOSPACE have rarely been corroborated with actual data, comparisons among management scenarios could still be comprehensive enough to screen different policies (Walters et al. 1999). In addition, ecological uncertainty can be incorporated through Monte Carlo simulations of trophic biomass flows using another tool of the ECOPATH software called ECORANGER (Christensen and Pauly 1992).

Another ecosystem approach, involving multiple trophic level models, is the habitat equivalency analysis (HEA; Julius 1999) that converts losses of production of multiple species to an energetically equivalent single trophic level. HEA has been used to estimate the scale of habitat restoration required to compensate for losses of organisms at multiple trophic levels resulting from habitat degradation or destruction (French McCay and Rowe 2003). Thus, it is not difficult to see that this approach explicitly addresses concerns associated with NNL. The use of food-chain transfers to assess changes in PC dates back to the 1960s, building upon food chain energetics (Slobodkin 1960; Odum 1971), and frequently relies on energy transfer efficiency at lower trophic levels to infer productivity changes at higher trophic levels following trophic cascades (Hashimoto et al. 1997; Yee et al. 2007). Studies of lower trophic dynamics have also been used to determine relationships between species richness and productivity (Yee et al. 2007) and have been coupled with water quality models (Saito et al. 2001) and water circulation models (Polovina et al. 2008) to assess ecosystem effects of changes in habitat quality.

Case studies

River and streams

Habitat science has a long history within flowing waters

(see Table 2). Hydrological methods, hydraulic rating curves, and habitat simulation models were derived from flowing systems and logically place an emphasis on the physical drivers of PC as rivers are defined by the physical aspects of their watersheds (Leopold et al. 1964; Hynes 1975; Vannote et al. 1980). Recently, in Canada, the impacts of proposed projects on fishes and fish habitat in a number of large rivers have been assessed under the FA. Three current examples give an overview of how PC is being assessed in these diverse situations: the Athabasca River, Churchill River, and Romaine River. We selected examples from large rivers (mean annual flow of about 300, 600, and 1700 $m^3 \cdot s^{-1}$ for Romaine, Athabasca, and Lower Churchill rivers, respectively) to demonstrate the challenges associated with large-scale assessments.

The Athabasca River in northern Alberta has recently been the subject of an extensive review related to water withdrawals associated with the oil and gas sector (Ohlson et al. 2010). Within this review, fish habitat considerations were analyzed by the instream flow incremental methodology (IFIM) process (Paul and Locke 2009). Local habitat use data for fishes was lacking, so habitat suitability curves were developed by expert opinion (see Paul and Locke 2009), augmented by local radiotelemetry investigations for a few species (Golder Associates Ltd. 2009). The IFIM process led to the evaluation of expected habitat loss for the species tested under various water withdrawal scenarios and identified two important areas for future research: (*i*) quantification of habitat–population links; and (*ii*) within Canada, the need for habitat research in the winter is paramount. Although these topics should be the subject of directed research, the Athabasca example provides an ideal scenario for an adaptive monitoring program to test predictions. However, in the context of the assessment of NNL of PC (Table 1), this process would score low on compliance given the focus on physical habitat change and assumed production–habitat relationships.

The lower section of the Churchill River in Labrador is currently under review in relation to a proposed project that would harness its remaining hydroelectric potential (Nalcor 2009). This project used a modification of the habitat evaluation procedure (U.S. Fish and Wildlife Service 1980, 1981) and an extensive fish capture database collected locally over an extended period of time (Ryan 1980; McCarthy et al. 2008) to generate life-stage-specific catch-based utilization indices (CUIs) (McCarthy et al. 2008). The resulting large number of CUIs, some of which still relied heavily on literature-derived information, were reduced to a manageable number by a guilding approach (McCarthy et al. 2008). These grouped CUIs could then be used to quantify the habitat proposed to be altered or destroyed by the project but could not make predictions in relation to future conditions as many of the locally derived habitat classifications would be changed after the project. Although using locally collected data reduced a number of uncertainties, many assumptions still had to be made, including uncertainties related to gear selectivity, habitat characterization, and temporal considerations, as all fish captures occurred under ice-free conditions.

A new hydro development (1550 MW capacity) is proposed for the Romaine River located on the north shore of the St. Lawrence River, Quebec. The proposal involves the construction of four dams that will transform two-thirds of the 300 km river into reservoirs. Anadromous Atlantic salmon utilize the lower 52 km of river, below an impassable natural waterfall. The PC of the river and proposed reservoir habitat were assessed using a biological (community-based) rather than a habitat-based approach (Bérubé et al. 2005; Smokorowski and Derbowka 2008), although the methods used to generate data did depend on habitat type: the main river, tributaries, lakes, and reservoirs (Smokorowski and Derbowka 2008). For all habitat types, fish production per unit area for each species was estimated using biomass density and body size as predictors and was scaled up as the product of unit area production and total surface area. Future PC of reservoirs was extrapolated from the current estimates of lake production, together with data from an existing Quebec reservoir, and scaling factors for projected reservoir surface areas. Methods for estimating fish density and production were based on a number of assumptions related to fish catches from different habitats (gear bias, catch efficiency, and others), and significantly, the estimates assumed a change in the fish community because of reservoir development (Smokorowski and Derbowka 2008).

Proposed approaches for assessing PC in the Romaine River are currently being reviewed. From a science perspective, the Romaine River case study is timely and important for two reasons: (*i*) as a test of the efficacy, limitations, and constraints of using a biological-based (production-based) approach; and (*ii*) the need for accepted science-based fish community targets to provide guidelines and context for predicting and modeling changes in fish production. Defining acceptable limits to changes in fish composition caused by large-scale changes in habitat (river to reservoir) is a key policy issue that applies not only to the Romaine, but also to many other watersheds undergoing development in Canada.

Lakes

Lake ecosystems provide a set of case studies to demonstrate the utility of habitat-based methods to measure PC. All of these examples would score relatively low on compliance as they rely heavily on literature-derived HSI and not any measure or index of biological production rate (Table 1). Results and application of habitat-based methods are initially discussed in the context of coastal areas of the Great Lakes and are then extended to inland lakes to contrast spatial scales.

MacNeil et al. (2008) analysed waterfront stabilization projects that altered fish habitat in the Great Lakes from 1997 to 2001. During this period, 53 projects that involved infilling (direct loss of habitat area ranging from 0 to 6000 m^2) were authorized under the FA (Section 35) (MacNeil et al. 2008). MacNeil et al. (2008) used the operational application of defensible methods, the habitat alteration assessment tool (HAAT), in retrospect to assess NNL of fish habitat for a subset of 18 infilling projects. The effect of infilling on fish habitat was represented by changes to suitable habitat supply for all fish species known to have access to the site, categorized by their thermal preference, feeding group, and life stage. By design, HAAT is based on seven components within the assessment framework outlined in Table 1. Significantly, the case study analyses showed that despite compensation and attempts to offset losses through enhancement of modified areas, most projects resulted in a net loss

of habitat PC. The analysis of infilling in the Great Lakes demonstrated the importance of using quantitative habitat inventories, suitability indices, and a science-based model (e.g., HAAT) to assess PC, to guide future mitigation, and to determine the quantity and quality of habitat needed for compensation. Although impacts were inferred from literature-derived HSI curves, scoring low on our compliance framework, this example demonstrates the importance of using an objective, quantitative approach to achieve even assumed NNL and improve application of the precautionary principle in light of uncertainty.

Fish habitat classification models were developed for Severn Sound (Georgian Bay) and Bay of Quinte (Lake Ontario) to help guide the management of fish habitat in these degraded areas (Minns et al. 1999*b*, 2006). Habitat inventories were assembled in a geographic information system (GIS) database, and suitability values for all habitats were developed, providing a relative measure of PC. The resulting habitat classification maps aided in identifying and protecting high suitability habitat and targeted restoration at low suitability areas, thereby, demonstrating the efficacy of fish habitat management plans (DFO 1986) as a tool for managing habitat at a regional scale.

For inland lakes, HAAT was used to predict the effects of habitat alteration on fish populations (Frezza and Minns 2002). Littoral habitat diversity was decreased (experiment manipulation) in three small (<25 ha) Canadian Shield lakes by removing coarse woody material (CWM) from 50% of the nearshore area (Kelso et al. 2001; Smokorowski et al. 2006). Additionally, in one of the lakes, a portion of the substrate was covered with geotextile. The HAAT analysis indicated that despite the experimental alterations, only minor changes in habitat supply (weighted suitable area) were expected to occur. However, there were limitations in applying the HAAT model to small shield lakes (CWM was considered to be poorly represented in the assessment framework; Frezza and Minns 2002). Also, the magnitude of the response depended on the spatial scale; the physical habitat manipulations were spatially limited at both the whole lake and littoral zone scale. Preliminary BACI (before–after control–impact) analysis of field studies of potential impacts to fishes or their food supply failed to show significant responses (Smokorowski et al. 2004, 2006).

Coastal marine areas

Construction activities in marine coastal areas (e.g., wharves, breakwaters, and similar facilities) are undertaken to improve access to nearby marine resources and sea lanes and to protect industrial and personal property onshore. Habitat quality of coastal fishes is altered both during and following construction of coastal structures (e.g., Sanger et al. 2004*a*, 2004*b*). Despite the need for scientifically defensible approaches, empirical studies of the ecological impacts of wharfs and breakwaters have been few and limited in scope. Here we review three ongoing studies on the eas. and west coasts of Canada, focusing on three topics: (*i*) succession of biota on introduced rocky structures; (*ii*) scientifically defensible decisions; and (*iii*) effect of footprint size.

In 2008, DFO and academic scientists were asked to provide advice on the productivity of breakwaters relative to natural rocky coasts in the southern Gulf of St. Lawrence. Well-established breakwaters (>15 years old) of similar construction (large boulders) to natural rocky shores are being compared with those younger in age. It is anticipated that biota found on breakwaters will be a function of the characteristics of the structure (e.g., material, size, shape, aspect with respect to shore) and how long the structure had been in place.

Estimating scientifically defensible gains and losses due to construction despite high background variability is a challenge. Vertical structures with associated rugosity are often productive and are considered desirable features of the coastal environment (e.g., Gregory and Anderson 1997) due to introduced edge effects (e.g., Sargent et al. 2006). However, the extent to which wharves and breakwaters self-compensate is rarely quantified. Scientists in Newfoundland are addressing this knowledge deficiency via a 10-year study (2007–2016) of a total of 18 sites (12 wharf–breakwater and six control locations) to quantify the habitat and fish productivity. Using a randomized intervention analysis (RIA) technique, this study will empirically quantify changes in habitat, fishes, and epibenthic macroinvertebrates associated with the wharves and breakwaters, providing proponents with analytical tools necessary to evaluate potential effects of other such developments and assisting habitat managers by guiding their mitigation and compensation decisions.

The scale of potential impact of wharves and breakwaters is broad, ranging from a few square metres to many kilometres, introducing a significant question: Are large developments merely linearly scaled versions of smaller ones or do they require a qualitatively different approach to compensation and mitigation? In British Columbia, responses to wharf and breakwater alterations are being measured through estimates of biotic community composition (taxa counts), ecological structure (functional grouping and biomass), species diversity, and physical and chemical alterations to address this question. The marine studies briefly described in this section represent three of the first quantitative evaluations testing and challenging the assumption that introduced rocky substrate and other wharf and breakwater developments have negative impacts compared with the unaltered habitats, status quo, and should be minimized. All approaches being used are measuring aspects of biological productivity and, if applied correctly, should be able to assess NNL following all required assessment components (Table 1).

Discussion

The broad question raised in the title of this paper is addressed by discussing a series of subquestions.

What role has productive capacity had in past fish habitat management decision-making?

Although much improvement in methods for assessing PC has occurred since 1986, application of these methods remains limited in the HMP (Minns 2001; Harper and Quigley 2005; Quigley and Harper 2006). DFO makes limited use of science-based quantitative assessment methods (Cudmore-Vokey et al. 2000; Lange et al. 2000). This outcome is due, in part, to the lack of generalization of results and simplification of data needs to facilitate applications (Cudmore-Vokey et al. 2000). Most of the methodologies reviewed here were not specifically framed as estimates or surrogates of PC and

very few directly address the assessment of NNL of PC. Most operational applications have used relative suitability methods. HMP decision-making with regard to NNL is often nonquantitative. Such decision-making is not reproducible, transparent, or amenable to consistency when delivered across the country. Furthermore, many of the streamlining improvements in program delivery (PoE, integrated risk assessment) have neglected to address the likelihood that cumulative net loss is still facilitated by the many referrals judged to have low or no risk.

What progress has been made with productive capacity methodologies?

This review demonstrated considerable progress in the development of scientific methods of estimating PC since the Canadian policy for fish habitat management was put in place (DFO 1986). Science has progressed from simplistic to ecologically sophisticated concepts, from static to dynamic evaluation, and from partial to complete surrogates of PC but has yet to bring forward a common basis for estimating PC in all habitat management contexts. The progress demonstrated that as with fisheries management and species-at-risk assessments, the use of mathematical models is inescapable in fish habitat management; though it may be feasible given adequate time and resources to estimate PC at a proposed development site, a model still has to be used to predict change after development by which net change can be assessed. Given the limited time and resource available for assessment typical of development activities driven by socioeconomic pressures, the baseline evaluation of sites is often incomplete.

The progression of methods reviewed represents a continuum of completeness with respect to estimating PC. Complete estimation of ecosystem productivity and how it responds to dynamic change comes closest to providing an assessment of the maximum productive potential, whereas commonly used approaches such as individual performance metrics (e.g., growth) or catch-per-unit-effort represent partial methods of estimating PC. Given the necessary socioeconomic constraints on predevelopment assessment for all but the largest development projects, fish habitat management decision-making must rely on simple approaches that keep the assessment costs at acceptable levels relative to the value assigned to the development's purpose. This situation requires (*i*) that the simple surrogate models of PC be well supported by scientific evidence, and (*ii*) that the precautionary principle (Hornbaker and Cullen 2003) be firmly applied in inverse proportion to the completeness of the methods and, hence, directly in proportion to the uncertainty associated with incompleteness. When proposing particular approaches to assessing net change, practitioners need to clearly state the incompleteness and uncertainties of the approach: the greater the limitations, the greater the need for caution, hence the need for compensation ratios greater than 1:1 when using the simplest methods to assess net change of PC.

Despite this flourishing of possible approaches to assessing PC, there is relatively little evidence of their widespread application in habitat management decision-making. The simpler approaches such as instream flow analysis and variants of the defensible methods model based on the U.S. Fish and Wildlife Service HSI suitability models are the most used and, frequently, only for larger projects. In-house documentation of these applications is limited, and relatively little evidence of their application is presented in the primary literature. Suitability assessment models are popular with proponents of developments and their consultants (C.K. Minns, personal observation), but often their applications are flawed. The methods reviewed above are often not framed specifically as practical tools for fish habitat management, making it difficult for front-line management staff to adopt or recommend them. A key conclusion is that scientists need to make greater efforts to render the results of their studies in the form of operational models and practitioners need to be encouraged to make greater use of these models.

What do the case studies reveal about fish habitat management?

Most of the case studies reviewed were large scale with respect to either the size of ecosystem or the number of sites being considered together. Most were also the subject of research efforts, either large-scale comparative studies or whole-system experiments. The large scale is characteristic of the application of scientific methodology requiring time, many replicate sites (both control and impacted), and the active involvement of researchers. These are features beyond consideration for practitioners looking at single-development projects. Many of the case studies are ongoing and the results have yet to appear in the primary literature. Small-scale single-site studies rarely appear in the primary literature, although the published literature on cumulative meta-analytic studies of various aspects of habitat modification based, in part, on such small-scale studies is increasing.

Most case studies involve the extensive application of simpler surrogate measures of PC. As a result, little evidence is being accumulated about the complex dynamic ecosystem processes occurring when habitats are changed. Such ecosystem studies, however, often provide the strongest evidence of how the whole ecosystem responds to habitat changes (cf. Minns et al. 1996*a*) and not just the user-preferred fish stocks.

The best opportunity to conduct case studies are real-world development projects, although there can be many challenges with regard to study design: insufficient sample numbers, predevelopment time, adequate controls, etc. Monitoring programs are often specified in HADD authorizations under the FA, but the monitoring designs are typically insufficient to detect significant effects (Bradford et al. 2005). New ways should be sought to gather useful evidence from the many developments that go ahead every year (i.e., effectiveness monitoring).

What is productive capacity?

The review of methodologies and case studies reveals the incompleteness of most of the attempts to measure PC, raising again the question perennially asked since the release of Canada's fish habitat policy, "What is productive capacity?" That this question is still asked may explain the slow uptake of PC methodologies by practitioners.

The conventional scientific history of the term recounted above does not show the complete story. Adding on the diverse views expressed by habitat management practitioners, proponents of development projects and their consultants, environmental protection advocates, fishers of all kinds, and the

general public, a spectrum of four definitions of PC emerge: (*i*) the sustainable harvest of preferred fishery species that can be practically achieved from the fish habitat; (*ii*) the sustainable total production of all fish species that can be practically realized from the fish habitat; (*iii*) the sustainable maximum production of all fish species that might be realized, without fetter or constraint, from the supporting habitat (ecosystem); and (*iv*) the sustainable maximum natural production of all biota that might be channelled into fish production, without fetter or constraint, from the supporting habitat (ecosystem).

The original Policy for the Management of Fish Habitat (DFO 1986) definition did not include any quantitative operationally defined terms, and hence, the meaning has remained somewhat dependent on the value systems of the beholders. Hence, the spectrum of opinions matches that of the wider philosophical debates about the protection, use, and domination of nature. However, the original definition clearly excludes the first two definitions, given that maximum potential productivity is the target based in the character of the habitat and not the fishes themselves. These definitions are all incomplete with respect to consideration of species or biodiversity questions and (or) the completeness and resilience of ecosystem structures and processes tied to ecological goods and services potentially produced by the ecosystems as a result of the presence of fishes. Hence, in the current language of ecosystem science, the fourth definition might be considered the closest fit to the intentions of those who framed the habitat policy as it makes habitat central but may be harder to define operationally. Most fishery scientists will likely prefer the third definition as it centres on the fishes and closely matches conventional operational definitions of production such as quoted earlier here from Minns (1997). Most likely, the third most closely approximates the fourth.

Recognizing the broader meaning of PC imposes a need for more research to be directed to showing the relationship between the structure and function of aquatic ecosystems and their potential fisheries productivity. It also reinforces the idea that fish habitat management has to become ecosystem-based management.

What is the connection between productive capacity and ecosystem-based management?

As approaches for assessing the PC of fish habitats have been evolving, so have the approaches to fisheries management. The need to consider the whole ecosystem, not just component parts, has gained greater recognition in recent decades. Although still strongly influenced by a long history of independent single-species assessment, fisheries are increasingly being managed with ecosystem-based approaches. Similar changes are beginning to be considered in fish habitat management and habitat science. For example, the availability of methods using lower trophic metrics is particularly evident in ongoing research, including coastal marine cases. In addition, the cumulative effects of multiple stressors (including, but beyond, habitat perturbations) are included in ecosystem assessment frameworks (Gavaris 2009). Of course, using ecosystem-based approaches will not be immediately feasible in many situations; therefore, the limitations of simpler, less complete methodologies need to be acknowledged when making decisions. A hierarchy of limitations is needed. For example, simple habitat-only methods cannot account for the biological feedbacks, interactions, and thresholds that are encountered in habitat–population or ecosystem methods and so assumptions should err in favour of avoiding net loss of PC.

Another consideration with ecosystem-based fish habitat management is the type of ecosystem approach being used. Like sustainable development, the phrase "ecosystem approach" has been co-opted for a wide range of methodologies, which may or may not involve science-based components. Unlike fisheries-focused approaches (Gavaris 2009), broad-based approaches often lack operationally defined metrics (Fitzsimmons 1996; Link 2002) and any bounding based on the biophysical constraints inherent in the ecosystem, e.g., maximum natural primary production rate and the portion that can be trophically transferred into fishable biomass (see, for example, Pauly and Christensen 1995).

Habitat management based on a broad scientific definition of PC should have a central role in ecosystem-based management, as the productive potential inherent in many habitat features are prerequisite for sustainable human use of aquatic resources, including fisheries. Although the science of fisheries management is highly evolved compared with those of habitat and ecosystem-based management, habitat science is still far ahead of ecosystem science as it is strongly embedded in fisheries science. The narrow focus on physical changes to habitats has undoubtedly restricted the growth of habitat science. Stressors such as climate change and lake acidification are considered beyond the scope of the habitat policy, although changes in habitat conditions due to such stressors can profoundly affect PC. Rather than replacing habitat management with loosely defined ecosystem-based management concepts, the various PC metrics reviewed here should be more widely applied to assessing NNL of PC in support of DFO's Policy for the Management of Fish Habitat (DFO 1986).

What are the essential elements for fish habitat management decision-making?

The comparative review of potential metrics and case studies indirectly helped highlight the features of a complete assessment of net change of PC. Complete assessments have an essential set of components (Table 1). The focus of habitat assessment of physical aspects of PC means that the fishes, their physical habitat requirements, and the availability of habitat constitute the primary essential components (1–6). The net change aspects of assessment are addressed in the comparison of scenarios and consideration of uncertainty and timing (7–8). Then, there are many abiotic and biotic factors (9–10) that may also influence or determine the outcome of physical habitat changes.

Many of the methodologies reviewed above contain some, but not all, of these essential features, although some components are often only implied. The limited attention given to the net change aspects of the Policy were reflected in the lack of effort applied to rendering research results as operational tools for practitioners. Simple methods usually lack features that are likely to further limit the amount of habitat that can be lost or modified before significant loss of PC occurs. The review of assessment approaches did indicate a

progression towards explicit consideration of more of the essential features presented here. Eventually, methods should be developed that take full account of all identified essentials. In the meantime, there is a need to appreciate the risks associated with methods that include few essentials: the fewer features used in a particular method, the greater the need to apply the precautionary principle (Hornbaker and Cullen 2003).

What are the conclusions?

Six key conclusions of this review are as follows: (*i*) the scope of potential surrogate metrics of PC has been expanded in recent years; (*ii*) application of the surrogate PC metrics needs to be conditioned by their degree of completeness and their inversely related levels of uncertainty; (*iii*) research-based PC metrics need to be incorporated into practical operational management assessment tools; (*iv*) as a component of the assessment tools, effort is needed to better quantify and communicate the link between habitat suitability and absolute units of fish production; (*v*) testing the links between surrogate metrics and true PC needs to be thoroughly evaluated as it is the surrogates that will be used routinely; and (*vi*) larger-scale studies beyond the scope of individual development projects are needed to fully calibrate tools for operational use, possibly via regulator–industry partnerships.

What is the way forward?

The growth of methods for measuring PC has not been confined to the fish habitat management arena. Production (growth and reproduction rates to match or exceed mortality rates) is a defining feature of biological systems. PC and the role of habitat is a central consideration for understanding and managing species-at-risk, invasive species, conservation of biodiversity, fisheries, and ecosystems. The knowledge needed to advance tools for measuring PC for habitat management will benefit from the expanding but complementary science needed to address these current and emerging management issues. This perspective emphasizes that the way forward includes three key components: (*i*) strong communication between managers and scientists to better facilitate the application and validation of measures of PC; (*ii*) targeted work to continue to build on and address the six conclusions listed previously, with increasing emphasis on dynamic models that integrate population responses to habitat supply characteristics; and (*iii*) incorporation of knowledge and techniques relevant to habitat science from other emerging issues that affect the productivity and biodiversity of aquatic habitat and ecosystems. Recently, DFO has moved towards addressing these components by creating two centers of expertise relevant to habitat science and management: (*i*) the Center of Expertise for Aquatic Habitat Research (CAHR); and (*ii*) the Center of Expertise on Hydropower Impacts on Fish and Fish Habitat (CHIF). Both of these virtual centers include a network of scientists, academics, and sector partners (i.e., industry) to promote fish habitat science efforts across Canada, ultimately to understand PC linkages and facilitate the efficient and sustainable use of Canada's natural resources within regulatory frameworks. DFO has further expanded its ability to achieve research objectives by partnering with the Natural Sciences and Engineering Research Council of Canada's HydroNet, a national research network to promote sustainable hydropower and healthy aquatic ecosystems. The network goals are to develop new knowledge and new tools to assess, minimize, and mitigate the effects of hydropower on fishes and their habitats, improve the decision-making process associated with hydropower operations, and reduce conflict among stakeholders. Together, these initiatives should continue to advance fish habitat science and its functional application in the decision-making process.

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References

Adams, S.M., Brown, A.M., and Goede, R.W. 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. Trans. Am. Fish. Soc. **122**(1): 63–73. doi:10.1577/1548-8659(1993)122<0063:AQHAIF>2.3.CO;2.

Ahmadi-Nedushan, B., St-Hilaire, A., Bérubé, M., Robichaud, É., Thiémonge, N., and Bobée, B. 2006. A review of statistical methods for the evaluation of aquatic habitat suitability for instream flow assessment. River Res. Appl. **22**(5): 503–523. doi:10.1002/rra.918.

Amara, R., Selleslagh, J., Billon, G., and Minier, C. 2009. Growth and condition of 0-group European flounder, *Platichthys flesus*, as indicator of estuarine habitat quality. Hydrobiologia, **627**(1): 87–98. doi:10.1007/s10750-009-9717-9.

Anderson, K.E., Paul, A.J., McCauley, E., Jackson, L.J., Post, J.R., and Nisbet, R.M. 2006. Instream flow needs in streams and rivers: the importance of understanding ecological dynamics. Front. Ecol. Environ. **4**(6): 309–318. doi:10.1890/1540-9295(2006)4[309:IFNISA]2.0.CO;2.

Baglinière, J.-L., Marchand, F., and Vauclin, V. 2005. Interannual changes in recruitment of the Atlantic salmon (*Salmo salar*) population in the River Oir (Lower Normandy, France): relationships with spawners and in-stream habitat. ICES J. Mar. Sci. **62**(4): 695–707. doi:10.1016/j.icesjms.2005.02.008.

Barbour, M.T., Stribling, J.B., and Karr, J.B. 1995. Multimetric approaches for establishing biocriteria and measuring biological condition. *In* Biological assessment criteria: tools for water resource planning and decision making. *Edited by* W.S. Davis and T.P. Simon. Lewis Publishers, Boca Raton, Florida. pp. 63–77.

Bélanger, G., and Rodríguez, M.A. 2002. Local movement as a measure of habitat quality in stream salmonids. Environ. Biol. Fishes, **64**(1/3): 155–164. doi:10.1023/A:1016044725154.

Benke, A.C. 1994. Edgardo Baldi Memorial Lecture: concepts and patterns of invertebrate production in running waters. Verh. Internat. Verein. Limnol. **25**: 15–38.

Bérubé, M., Verdon, R., Durocher, G., and Guay, J.-C. 2005. A comprehensive framework for assessing habitat changes in fish habitat productive capacity resulting from large hydroelectric projects. Can. Sci. Advis. Sec. Res. Doc. 2005/051.

Bonada, N., Prat, N., Resh, V.H., and Statzner, B. 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annu. Rev. Entomol. **51**(1): 495–523. doi:10.1146/annurev.ento.51.110104.151124. PMID:16332221.

Bradford, M.J., Korman, J., and Higgins, P.S. 2005. Using confidence intervals to estimate the response of salmon populations

(*Oncorhynchus* spp.) to experimental habitat alterations. Can. J. Fish. Aquat. Sci. **62**(12): 2716–2726. doi:10.1139/f05-179.

Brandt, S.B., and Kirsch, J. 1993. Spatially explicit models of striped bass growth potential in Chesapeake Bay. Trans. Am. Fish. Soc. **122**(5): 845–869. doi:10.1577/1548-8659(1993)122<0845:SEMOSB>2.3.CO;2.

Caissie, D., and El-Jabi, N. 1995. Comparison and regionalization of hydrologically based instream flow techniques in Atlantic Canada. Can. J. Civ. Eng. **22**(2): 235–246. doi:10.1139/l95-033.

Caswell, H. 2001. Matrix population models: construction, analysis, and interpretation. Sinauer Associates, Sunderland, Massachusetts.

Christensen, V., and Pauly, D. 1992. The ECOPATH II — a software for balancing steady-state ecosystem models and calculating network characteristics. Ecol. Modef. **61**(3–4): 169–185. doi:10.1016/0304-3800(92)90016-8.

Chu, C., Minns, C.K., and Mandrak, N.E. 2003. Comparative regional assessment of factors impacting freshwater fish biodiversity in Canada. Can. J. Fish. Aquat. Sci. **60**(5): 624–634. doi:10.1139/f03-048.

Clarke, K.D., and Scruton, D.A. 2002. Evaluating efforts to increase salmonid productive capacity through habitat enhancement in the low diversity/production systems of Newfoundland, Canada. *In* Proceedings of the 13th International Salmonid Habitat Enhancement Workshop, Westport, Ireland, 16–19 September 2002. Irish Freshwater Ecology and Management Series, Central Fisheries Board, Dublin, Ireland. pp. 160–182.

Clarke, K., Pratt, T.C., Randall, R.C., Scruton, D.A., and Smokorowski, K.E. 2008. Validation of the flow management pathway: effects of altered flow on fish habitat and fishes downstream from a hydropower dam. Can. Tech. Rep. Fish. Aquat. Sci. 2784..

Copeland, T., Murphy, B.R., and Ney, J.J. 2008. Interpretation of relative weight in three populations of wild bluegills: a cautionary tale. N. Am. J. Fish. Manage. **28**(2): 368–377. doi:10.1577/M05-171.1.

Cote, D. 2007. Measurements of salmonid population performance in relation to habitat in eastern Newfoundland streams. J. Fish Biol. **70**(4): 1134–1147. doi:10.1111/j.1095-8649.2007.01384.x.

Cowan, J.H., Jr., Rose, K.A., and DeVries, D.R. 2000. Is density-dependent growth in young-of-the-year fishes a question of critical weight? Rev. Fish Biol. Fish. **10**(1): 61–89. doi:10.1023/A:1008932401381.

Crowder, L.B., Lyman, S.J., Figueira, W.F., and Priddy, J. 2000. Source–sink population dynamics and the problem of sitting marine reserves. Bull. Mar. Sci. **66**: 799–820.

Crozier, L.G., Zabel, R.W., Hockersmith, E.E., and Achord, S. 2010. Interacting effects of density and temperature on body size in multiple populations of Chinook salmon. J. Anim. Ecol. **79**(2): 342–349. doi:10.1111/j.1365-2656.2009.01641.x. PMID: 20002859.

Cudmore-Vokey, B.C., Lange, M., and Minns, C.K. 2000. Database documentation and critical review of national habitat compensation literature. Can. Manuscr. Rep. Fish. Aquat. Sci. 2526..

de Kerckhove, D.T., Smokorowski, K.E., and Randall, R.G. 2008. A primer on fish habitat models. Can. Tech. Rep. Fish. Aquat. Sci. 2817..

Deegan, L.A., Finn, J.T., Ayvazian, S.G., Ryder-Kieffer, C.A., and Buonaccorsi, J. 1997. Development and validation of an estuarine biotic integrity index. Estuaries, **20**(3): 601–617. doi:10.2307/1352618.

Dolinsek, I.J., Biron, P.M., and Grant, J.W.A. 2007. Assessing the effect of visual isolation on the population density of Atlantic salmon (*Salmo salar*) using GIS. River Res. Appl. **23**(7): 763–774. doi:10.1002/rra.1024.

Downing, J.A., Plante, C., and Lalonde, S. 1990. Fish production correlated with primary production, not the morphoedaphic index. Can. J. Fish. Aquat. Sci. **47**(10): 1929–1936. doi:10.1139/f90-217.

Eby, L.A., Crowder, L.B., McClellan, C.M., Peterson, C.H., and Powers, M.J. 2005. Habitat degradation from intermittent anoxia: impacts on demersal fishes. Mar. Ecol. Prog. Ser. **291**: 249–261. doi:10.3354/meps291249.

Englert, T.L., Wells, A.W., and Norris, R.A. 2000. Incorporation of changes in habitat quantity and quality into density-dependent population models. Environ. Sci. Policy, **3**(Suppl. 1): 451–458. doi:10.1016/S1462-9011(00)00057-5.

Fausch, K.D., Hawkes, C.L., and Parsons, M.G. 1988. Models that predict the standing crop of stream fish from habitat variables. USDA Forest Service, Portland, Oregon, Gen. Tech. Rep. PNW-GTR-213.

Fisheries and Oceans Canada. 1986. The Department of Fisheries and Oceans Policy for the Management of Fish Habitat. Communications Directorate, Fisheries and Oceans Canada, Ottawa, Ontario, DFO/3524..

Fisheries and Oceans Canada. 2004. Science technology transfer workshop — science contributions towards improving fish habitat management. Can. Advis. Sec. Proc. Ser. 2004/010.

Fisheries and Oceans Canada. 2007*a*. A new ecosystem science framework in support of integrated management. Communications Directorate, Fisheries and Oceans Canada, Ottawa, Ontario, DFO/2007-1296.

Fisheries and Oceans Canada. 2007*b*. Documenting habitat use of species at risk and quantifying habitat quality. DFO Can. Sci. Advis. Sec. Sci. Rep. 2007/038.

Fitzsimmons, A.K. 1996. Sound policy or smoke and mirrors: does ecosystem management make sense? Water Resour. Bull. **32**(2): 217–227.

French McCay, D.P., and Rowe, J.J. 2003. Habitat restoration as mitigation for lost production at multiple trophic levels. Mar. Ecol. Prog. Ser. **264**: 233–247. doi:10.3354/meps264233.

Frezza, T., and Minns, C.K. 2002. Assessing fish habitat supply and potential responses to habitat manipulation in small Canadian Shield lakes. Can. Manuscr. Rep. Fish. Aquat. Sci. 2599.

Gavaris, S. 2009. Fisheries management planning and support for strategic and tactical decisions in an ecosystem approach context. Fish. Res. **100**(1): 6–14. doi:10.1016/j.fishres.2008.12.001.

Gibson, A.J.F. 2006. Population regulation in eastern Canadian Atlantic salmon (*Salmo salar*) populations. Can. Sci. Advis. Sec. Res. Doc. 2006/016.

Gilliers, C., Le Pape, O., Desaunay, Y., Morin, J., Guerault, D., and Amara, R. 2006. Are growth and density quantitative indicators of essential fish habitat quality? An application of the common sole, *Solea solea*, nursery grounds. Estuar. Coast. Shelf Sci. **69**(1–2): 96–106. doi:10.1016/j.ecss.2006.02.006.

Goede, R.W., and Barton, B.A. 1990. Organismic indices of an autopsy-based assessment as indicators of health and condition of fish. Am. Fish. Soc. Symp. **8**: 93–108.

Golder Associates Ltd. 2009. Summary of the lower Athabasca River fish habitat suitability criteria workshop (April 22 to 23, 2009). Cumulative Environmental Management Association (CEMA), Fort McMurray, Alberta. Available at http://cemaonline.ca.

Goodchild, G.A. 2004. Fish habitat is everyone's business, Canada's fish habitat management programme. Fish. Manag. Ecol. **11**(3–4): 277–281. doi:10.1111/j.1365-2400.2004.00409.x.

Gorman, A.M., Gregory, R.S., and Schneider, D.C. 2009. Eelgrass patch size and proximity to the patch edge affect predation risk of recently settled age 0 cod (*Gadus*). J. Exp. Mar. Biol. Ecol. **371**(1): 1–9. doi:10.1016/j.jembe.2008.12.008.

Gregory, R.S., and Anderson, J.T. 1997. Substrate selection and use of protective cover by juvenile Atlantic cod (*Gadus morhua*) in

inshore waters of Newfoundland. Mar. Ecol. Prog. Ser. **146**: 9–20. doi:10.3354/meps146009.

Grubaugh, J.W., Wallace, J.B., and Houston, E.S. 1997. Production of benthic macroinvertebrate communities along a Southern Appalachian river continuum. Freshw. Biol. **37**: 581–596. doi:10.1046/j.1365-2427.1997.d01-578.x.

Guay, J.C., Boisclair, D., Leclerc, M., and Lapointe, M. 2003. Assessment of the transferability of biological habitat models for Atlantic salmon parr (*Salmo salar*). Can. J. Fish. Aquat. Sci. **60**(11): 1398–1408. doi:10.1139/f03-120.

Harper, D.J., and Quigley, J.T. 2005. No net loss of fish habitat: a review and analysis of habitat compensation in Canada. Environ. Manage. **36**(3): 343–355. doi:10.1007/s00267-004-0114-x. PMID: 16082569.

Harvey, B.C., and Railsback, S.F. 2007. Estimating multi-factor cumulative effects on fish populations with an individual-based model. Fisheries (Bethesda), **32**(6): 292–298. doi:10.1577/1548-8446(2007)32[292:EFCWEO]2.0.CO;2.

Harvey, B.C., and Stewart, A.J. 1991. Fish size and habitat depth relationships in headwater streams. Oecologia (Berl.), **87**(3): 336–342. doi:10.1007/BF00634588.

Harvey, B.C., White, J.L., and Nakamoto, R.J. 2005. Habitat-specific biomass, survival and growth of rainbow trout (*Oncorhynchus mykiss*) during summer in a small coastal stream. Can. J. Fish. Aquat. Sci. **62**(3): 650–658. doi:10.1139/f04-225.

Hashimoto, H., Hashimoto, T., Matsuda, O., Tada, K., Tamai, K., Uye, S., and Yamamoto, T. 1997. Biological productivity of lower trophic levels of the Seto Inland Sea. *In* Sustainable development in the Seto Inland Sea, Japan — from the viewpoint of fisheries. *Edited by* T. Okaichi and T. Yanagi. Terra Scientific Publishing Company, Tokyo, Japan. pp. 15–58.

Hayes, D.B., Ferreri, C.P., and Taylor, W.W. 1996. Linking fish habitat to their population dynamics. Can. J. Fish. Aquat. Sci. **53**(Suppl. 1): 383–390. doi:10.1139/f95-273.

Hayes, D., Jones, M., Lester, N., Chu, C., Doka, S., Netto, J., Stockwell, J., Thompson, B., Minns, C.K., Shuter, B., and Collins, N. 2009. Linking fish population dynamics to habitat conditions: insights from the application of a process-oriented approach to several Great Lakes species. Rev. Fish Biol. Fish. **19**(3): 295–312. doi:10.1007/s11160-009-9103-8.

Heggenes, J., Saltveit, S.J., Bird, D., and Grew, R. 2002. Static habitat partitioning and dynamic selection by sympatric young Atlantic salmon and brown trout in south-west England streams. J. Fish Biol. **60**(1): 72–86. doi:10.1111/j.1095-8649.2002.tb02388.x.

Holm, C.F., Armstrong, J.D., and Gilvear, D.J. 2001. Investigating a major assumption of predictive instream habitat models: is water velocity preference of juvenile Atlantic salmon independent of discharge? J. Fish Biol. **59**(6): 1653–1666. doi:10.1111/j.1095-8649.2001.tb00228.x.

Honea, J.M., Jorgensen, J.C., McClure, M.M., Cooney, T.D., Engie, K., Holzer, D.M., and Hilborn, R. 2009. Evaluating habitat effects on population status: influence of habitat restoration on spring-run Chinook salmon. Freshw. Biol. **54**(7): 1576–1592. doi:10.1111/j.1365-2427.2009.02208.x.

Hornbaker, M.H., and Cullen, A. 2003. The precautionary principle in practice: applying the Ashford framework to technological risk. Hum. Ecol. Risk Assess. **9**(3): 789–810. doi:10.1080/713609967.

Hubert, W.A., and Guenther, P.M. 1992. Non-salmonid fishes and morphoedaphic features affect abundance of trouts in Wyoming reservoirs. Northwest Sci. **66**: 224–228.

Humston, R., Olson, D.B., and Ault, J.S. 2004. Behavioral assumptions in models of fish movement and their influence on population dynamics. Trans. Am. Fish. Soc. **133**(6): 1304–1328. doi:10.1577/T03-040.1.

Hunt, R.L. 1974. Long term production by brook trout in Lawrence Creek during eleven successive years. Wis. Dep. Nat. Res. Tech. Bull. 82.

Hynes, H.B.N. 1975. The stream and its valley. Verh. Internat. Verein. Limnol. **19**: 1–15.

Jackson, D.A., and Harvey, H.H. 1997. Qualitative and quantitative sampling of lake fish communities. Can. J. Fish. Aquat. Sci. **54**(12): 2807–2813. doi:10.1139/f97-182.

Jackson, D.A., Harvey, H.H., and Somers, K.M. 1990. Ratios in aquatic sciences: statistical shortcomings with mean depth and the morphoedaphic index. Can. J. Fish. Aquat. Sci. **47**(9): 1788–1795. doi:10.1139/f90-203.

Jenkins, R.M., and Oglesby, R. 1982. The morphoedaphic index and reservoir fish production. Trans. Am. Fish. Soc. **111**(2): 133–140. doi:10.1577/1548-8659(1982)111<133:TMIAPT>2.0.CO;2.

Jensen, D.W., Steel, E.A., Fullerton, A.H., and Pess, G.R. 2009. Impact of fine sediment on egg-to-fry survival of Pacific salmon: a meta-analysis of published studies. Rev. Fish. Sci. **17**(3): 348–359. doi:10.1080/10641260902716954.

Johnson, D.W. 2007. Habitat complexity modifies post-settlement mortality and recruitment dynamics of a marine fish. Ecology, **88**(7): 1716–1725. doi:10.1890/06-0591.1. PMID:17645018.

Jones, N.E., and Tonn, W.M. 2004. Enhancing productive capacity in the Canadian Arctic: assessing the effectiveness of instream habitat structures in habitat compensation. Trans. Am. Fish. Soc. **133**(6): 1356–1365. doi:10.1577/T03-136.1.

Jones, M.L., Randall, R.G., Hayes, D., Dunlop, W., Imhof, J., Lacroix, G., and Ward, N.J.R. 1996. Assessing the ecological effects of habitat change: moving beyond productive capacity. Can. J. Fish. Aquat. Sci. **53**(Suppl. 1): 446–457. doi:10.1139/f96-013.

Jones, N.E., Tonn, W.M., Scrimgeour, G.J., and Katopodis, C. 2003. Productive capacity of an artificial stream in the Canadian Arctic: assessing the effectiveness of fish habitat compensation. Can. J. Fish. Aquat. Sci. **60**(7): 849–863. doi:10.1139/f03-074.

Jones, N.E., Scrimgeour, G.J., and Tonn, W.M. 2008. Assessing the effectiveness of a constructed Arctic stream using multiple biological attributes. Environ. Manage. **42**(6): 1064–1076. doi:10.1007/s00267-008-9218-z. PMID:18839240.

Julius, B. 1999. Discounting and the treatment of uncertainty in natural resource damage assessment. NOAA Damage Assessment and Restoration Program, Silver Spring, Maryland, Technical Paper 99-1.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecol. Appl. **1**(1): 66–84. doi:10.2307/1941848.

Kashiwai, M. 1995. History of carrying capacity concept as in index of ecosystem productivity. Bull. Hokkaido Natl. Fish. Res. Inst. **59**: 81–101.

Katopodis, C. 2003. Case studies of instream flow modelling for fish habitat in Canadian Prairie rivers. Can. Water Resour. J. **28**(2): 199–216. doi:10.4296/cwrj2802199.

Kelso, J.R.M., Steedman, R.J., Gunn, J.M., Smokorowski, K.E., Lester, N.P., Cole, W.G., Minns, C.K., and Mills, K.H. 2001. A framework for the advancement of aquatic science — lake habitat experiments as an example. Aquat. Ecosyst. Health Manage., **4**(4): 453–461. doi:10.1080/146349801317276116.

Khalil, M.T. 1997. Prediction of fish yield and potential productivity from limnological data in Lake Borollus, Egypt. Int. J. Salt Lake Res. **6**(4): 323–330. doi:10.1007/BF02447914.

Kilgour, B.W., Neary, J., Ming, D., and Beach, D. 2005. Preliminary investigations of the use and status of instream-flow-needs methods in Ontario with specific reference to application with hydroelectric developments. Can. Manuscr. Rep. Fish. Aquat. Sci. 2723.

Knapp, R.A., Vredenburg, V.T., and Matthews, K.R. 1998. Effects of stream channel morphology on golden trout spawning habitat and recruitment. Ecol. Appl. **8**(4): 1104–1117. doi:10.1890/1051-0761(1998)008[1104:EOSCMO]2.0.CO;2.

Laë, R., Lek, S., and Moreau, J. 1999. Predicting fish yield of African lakes using neural networks. Ecol. Model. **120**(2–3): 325–335. doi:10.1016/S0304-3800(99)00112-X.

Lane, J.A., Portt, C.B., and Minns, C.K. 1996*a*. Nursery habitat characteristics of Great Lakes fishes. Can. Manuscr. Rep. Fish. Aquat. Sci. 2338.

Lane, J.A., Portt, C.B., and Minns, C.K. 1996*b*. Adult habitat characteristics of Great Lakes fishes. Can. Manuscr. Rep. Fish. Aquat. Sci. 2358.

Lane, J.A., Portt, C.B., and Minns, C.K. 1996*c*. Spawning habitat characteristics of Great Lakes fishes. Can. Manuscr. Rep. Fish. Aquat. Sci. 2368.

Lange, M., Cudmore-Vokey, B.C., and Minns, C.K. 2000. Habitat compensation case study analysis. Can. Manuscr. Rpt. Fish. Aquat. Sci. 2576.

Larkin, P.A. 1996. Concepts and issues in marine ecosystem management. Rev. Fish Biol. Fish. **6**(2): 139–164. doi:10.1007/BF00182341.

Laurel, B.J., Gregory, R.S., and Brown, J.A. 2003. Predator distribution and habitat patch area determine predation rates on age-0 juvenile cod *Gadus* spp. Mar. Ecol. Prog. Ser. **251**: 245–254. doi:10.3354/meps251245.

Lek, S., Delacoste, M., Baran, P., Dimopoulos, I., Lauga, J., and Aulagnier, S. 1996. Application of neural networks to modelling nonlinear relationships in ecology. Ecol. Model. **90**(1): 39–52. doi:10.1016/0304-3800(95)00142-5.

Leopold, L.B., Wolman, M.G., and Miller, J.P. 1964. Fluvial processes in geomorphology. W.H. Freeman, San Francisco, California.

Levin, P.S., and Stunz, G.W. 2005. Habitat triage for exploited fishes: can we identify essential "essential fish habitat"? Estuar. Coast. Shelf Sci. **64**(1): 70–78. doi:10.1016/j.ecss.2005.02.007.

Link, J.S. 2002. What does ecosystem-based fisheries management mean? Fisheries (Bethesda), **27**: 18–21.

Lobón-Cerviá, J. 2007. Numerical changes in stream-resident brown trout (*Salmo trutta*): uncovering the roles of density-dependent and density-independent factors across space and time. Can. J. Fish. Aquat. Sci. **64**(10): 1429–1447. doi:10.1139/f07-111.

Lomnicki, A. 1988. Population ecology of individuals. Monographs in Population Biology 25, Princeton University Press, Princeton, New Jersey.

Long, J.M., and Walker, D.J. 2005. Small scale application and assessment of a index of biotic integrity for a large boreal river. Hydrobiologia, **544**(1): 177–187. doi:10.1007/s10750-005-0543-4.

Lucas, M.G., and Baras, E. 2000. Methods for studying spatial behaviour of freshwater fishes in the natural environment. Fish Fish. **1**: 283–316.

MacNeil, J.E., Murphy, S., Ming, D., and Minns, C.K. 2008. Analysis of infilling projects affecting fish habitat in the Great Lakes (1997–2001). Can. Manuscr. Rep. Fish. Aquat. Sci. 2840.

Mäki-Petäys, A., Huusko, A., Erkinaro, J., and Muotka, T. 2002. Transferability of habitat suitability criteria of juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **59**(2): 218–228. doi:10.1139/f01-209.

Mangel, M. 2000. Trade-offs between fish habitat and fishing mortality and the role of reserves. Bull. Mar. Sci. **66**: 663–674.

Mason, D.M., Goyke, A.P., and Brandt, S.B. 1995. A spatially explicit bioenergetics measure of habitat quality for adult salmonines: comparison between Lakes Michigan and Ontario. Can. J. Fish. Aquat. Sci. **52**(7): 1572–1583. doi:10.1139/f95-750.

McCarthy, J., LeDrew, B.R., and LeDrew, L.J. 2008. A framework for aquatic habitat classification for large northern ecosystems: application to the proposed Churchill River Power Project, Churchill River Labrador, Canada. Am. Fish. Soc. Symp. **49**: 1041–1057.

McMahon, T.E., and Matter, W.J. 2006. Linking habitat selection, emigration and population dynamics of freshwater fishes: a synthesis of ideas and approaches. Ecol. Freshwat. Fish, **15**(2): 200–210. doi:10.1111/j.1600-0633.2006.00130.x.

Meng, L., Orphanides, C.D., and Powell, J.C. 2002. Use of a fish index to assess habitat quality in Narragansett Bay, Rhode Island. Trans. Am. Fish. Soc. **131**(4): 731–742. doi:10.1577/1548-8659(2002)131<0731:UOAFIT>2.0.CO;2.

Minns, C.K. 1997. Quantifying "no net loss" of productivity of fish habitats. Can. J. Fish. Aquat. Sci. **54**(10): 2463–2473. doi:10.1139/cjfas-54-10-2463.

Minns, C.K. 2001. Science for freshwater fish and habitat management in Canada: current status. Aquat. Ecosyst. Health Manage. **4**(4): 423–436. doi:10.1080/146349801317276099.

Minns, C.K. 2003. An area-per-individual (API) model for estimating critical habitat requirements in aquatic species-at-risk. Fisheries and Oceans Canada, Ottawa, Ontario, Can. Sci. Advis. Sec. Res. Doc. 2003/074.

Minns, C.K. 2006. Compensation ratios needed to offset timing effects of losses and gains and achieve no net loss of productive capacity of fish habitat. Can. J. Fish. Aquat. Sci. **63**(5): 1172–1182. doi:10.1139/f06-025.

Minns, C.K., and Moore, J.E. 2003. Assessment of net change of productive capacity of fish habitats: the role of uncertainty and complexity in decision-making. Can. J. Fish. Aquat. Sci. **60**(1): 100–116. doi:10.1139/f02-168.

Minns, C.K., and Nairn, R.B. 1999. Defensible methods: applications of a procedure for assessing developments affecting littoral fish habitat on the lower Great Lakes. *In* Aquatic restoration in Canada. *Edited by* T.P. Murphy and M. Munawar. Backhuys Publishers, Leiden, the Netherlands. pp. 15–35.

Minns, C.K., Cairns, V.W., Randall, R.G., and Moore, J.E. 1994. An index of biotic integrity (IBI) for fish assemblages in the littoral zone of Great Lakes' Areas of Concern. Can. J. Fish. Aquat. Sci. **51**(8): 1804–1822. doi:10.1139/f94-183.

Minns, C.K., Kelso, J.R.M., and Randall, R.G. 1996*a*. Detecting the response of fish to habitat alterations in freshwater ecosystems. Can. J. Fish. Aquat. Sci. **53**(Suppl. 1): 403–414. doi:10.1139/f95-262.

Minns, C.K., Randall, R.G., Moore, J.E., and Cairns, V.W. 1996*b*. A model simulating the impact of habitat supply limits on northern pike, *Esox lucius*, in Hamilton Harbour, Lake Ontario. Can. J. Fish. Aquat. Sci. **53**(Suppl. 1): 20–34. doi:10.1139/f95-258.

Minns, C.K., Doka, S.E., Bakelaar, C.N., Brunette, P.C.E., and Schertzer, W.M. 1999*a*. Identifying habitats essential for pike *Esox lucius* L. in the Long Point Region of Lake Erie: a suitability supply approach. Am. Fish. Soc. Symp. **22**: 363–382.

Minns, C.K., Brunette, P., Stoneman, M., Sherman, K., Craig, R., Portt, C., and Randall, R.G. 1999*b*. Development of a fish habitat classification model for littoral areas of Severn Sound, Georgian Bay, a Great Lakes' area of concern. Can. Manuscr. Rep. Fish. Aquat. Sci. 2490.

Minns, C.K., Moore, J.E., Stoneman, M., and Cudmore-Vokey, B. 2001. Defensible methods of assessing fish habitat: lacustrine habitats in the Great Lakes Basin — conceptual basis and approach using a Habitat Suitability Matrix (HSM) method. Can. Manuscr. Rep. Fish. Aquat. Sci. 2559.

Minns, C.K., Bernard, A., Bakelaar, C.N., and Ewaschuk, M. 2006. A fish habitat classification model for the upper and lower sections of

Bay of Quinte, Lake Ontario. Can. Manuscr. Rep. Fish. Aquat. Sci. 2748.

Morris, W.F., and Doak, D.F. 2002. Quantitative conservation biology: theory and practice of population viability analysis. Sinauer Associates, Sunderland, Massachusetts.

Moussalli, E., and Hilborn, R. 1986. Optimal stock size and harvest rate in multistage life history models. Can. J. Fish. Aquat. Sci. **43**(1): 135–141. doi:10.1139/f86-014.

Munkittrick, K.R., and Dixon, D.G. 1989. A holistic approach to ecosystem health assessment using fish population characteristics. Hydrobiologia, **188–189**(1): 123–135. doi:10.1007/BF00027777.

Nalcor Energy. 2009. Lower Churchill Hydroelectric Generation Project: Environmental Impact Statement. Nalcor Energy, St. John's, Newfoundland. Available at http://www.nalcorenergy.com/environmental-assessment-generation.asp.

Niemi, G.J., and McDonald, M.E. 2004. Application of ecological indicators. Annu. Rev. Ecol. Evol. Syst. **35**(1): 89–111. doi:10.1146/annurev.ecolsys.35.112202.130132.

Nykänen, M., and Huusko, A. 2004. Transferability of habitat preference criteria for larval European grayling (*Thymallus thymallus*). Can. J. Fish. Aquat. Sci. **61**(2): 185–192. doi:10.1139/f03-156.

O'Shea, D.T. 1995. Estimating minimum instream flow requirements for Minnesota streams from hydrologic data and watershed characteristics. N. Am. J. Fish. Manage. **15**(3): 569–578. doi:10.1577/1548-8675(1995)015<0569:EMIFRF>2.3.CO;2.

Odum, E.P. 1971. Fundamentals of ecology. 5th ed. W.B. Saunders, Philadelphia, Pennsylvania.

Ohlson, D., Long, G., and Hatfield, T. 2010. Phase 2 Framework Committee Report. Cumulative Environmental Management Association (CEMA), Fort McMurray, Alberta. Available at http://cemaonline.ca/cema-recommendations/phase-ii-water-management-framework.html.

Osenberg, C.W., Schmitt, R.J., Holbrook, S.J., Abu-Saba, K.E., and Flegal, A.R. 1994. Detection and environmental impacts: natural variability, effect size, and power analysis. Ecol. Appl. **4**(1): 16–30. doi:10.2307/1942111.

Parasiewicz, P. 2001. MesoHABSIM: a concept for application of instream flow models in river restoration planning. Fisheries (Bethesda), **26**(9): 6–13. doi:10.1577/1548-8446(2001)026<0006:M>2.0.CO;2.

Parasiewicz, P. 2007*a*. The MesoHABSIM model revisited. River Res. Appl. **23**(8): 893–903. doi:10.1002/rra.1045.

Parasiewicz, P. 2007*b*. Using MesoHABSIM to develop reference habitat template and ecological management scenarios. River Res. Appl. **23**(8): 924–932. doi:10.1002/rra.1044.

Parasiewicz, P., and Walker, J.D. 2007. Comparison of MesoHABSIM with two microhabitat models (PHABSIM and HARPHA). River Res. Appl. **23**(8): 904–923. doi:10.1002/rra.1043.

Parken, C.K., McNicol, R.E., and Irvine, J.R. 2006. Habitat-based methods to estimate escapement goals for data-limited Chinook salmon stocks in British Columbia, 2004. Can. Sci. Advis. Sec. Res. Doc. 2006/083.

Paul, A.J., and Locke, A. 2009. Evaluation criteria for flow alterations in the Lower Athabasca River — Fish Habitat. Phase 2 Framework Committee Report, Vol. 2, Technical Appendices. Cumulative Environmental Management Association (CEMA), Fort McMurray, Alberta. Available at http://cemaonline.ca/cema-recommendations/phase-ii-water-management-framework.html.

Pauly, D., and Christensen, V. 1995. Primary production required to sustain global fisheries. Nature, **374**(6519): 255–257. doi:10.1038/374255a0.

Pearson, M.P., Quigley, J.T., Harper, D.J., and Galbraith, R.V. 2005. Monitoring and assessment of fish habitat compensation and stewardship projects: study design, methodology an example case studies. Can. Manuscr. Rep. Fish. Aquat. Sci. 2729.

Pihl, L., Modin, J., and Wennhage, H. 2005. Relating plaice (*Pleuronectes platessa*) recruitment to deteriorating habitat quality: effects of macroalgal blooms in coastal nursery grounds. Can. J. Fish. Aquat. Sci. **62**(5): 1184–1193. doi:10.1139/f05-023.

Pitcher, T.J., Buchary, E.A., and Hutton, T. 2002. Forecasting the benefits of no-take human-made reefs using spatial ecosystems simulation. ICES J. Mar. Sci. **59**: S17–S26. doi:10.1006/jmsc.2002.1185.

Polovina, J.J., Chai, F., Howell, E.A., Kobayashi, D.R., Shi, L., and Chao, Y. 2008. Ecosystem dynamics at a productivity gradient: a study of the lower trophic dynamics around the northern atolls in the Hawaiian Archipelago. Prog. Oceanogr. **77**(2–3): 217–224. doi:10.1016/j.pocean.2008.03.011.

Portt, C.B., Coker, G.A., Ming, D.L., and Randall, R.G. 2006. A review of fish sampling methods commonly used in Canadian freshwater habitats. Can. Tech. Rep. Fish. Aquat. Sci. 2604.

Pratt, T. 2004. Habitat-specific production rate estimates from 5 Canadian shield lakes. Can. Sci. Advis. Sec. Res. Doc. 2004/085.

Quigley, J.T., and Harper, D.J. 2006. Effectiveness of fish habitat compensation in Canada in achieving no net loss. Environ. Manage. **37**(3): 351–366. doi:10.1007/s00267-004-0263-y. PMID:16456631.

Quinn, T.J., II, and Collie, J.S. 2005. Sustainability in single-species population models. Philos. Trans. R. Soc. B, **360**(1453): 147–162. doi:10.1098/rstb.2004.1577. PMID:15713594.

Railsback, S.F., Stauffer, H.B., and Harvey, B.C. 2003. What can habitat preference models tell us? Tests using a virtual trout population. Ecol. Appl. **13**(6): 1580–1594. doi:10.1890/02-5051.

Randall, R.G. 2003. Fish productivity and habitat productive capacity: definitions, indices, units of field measurement, and a need for standardized terminology. Can. Sci. Advis. Sec. Res. Doc. 2003/061.

Randall, R.G., and Minns, C.K. 2000. Use of fish production per unit biomass ratios for measuring the productive capacity of fish habitats. Can. J. Fish. Aquat. Sci. **57**(8): 1657–1667. doi:10.1139/f00-103.

Randall, R.G., and Minns, C.K. 2002. Comparison of a habitat productivity index and an index of biotic integrity for measuring the productive capacity of fish habitat in nearshore areas of the Great Lakes. J. Great Lakes Res. **28**(2): 240–255. doi:10.1016/S0380-1330(02)70580-7.

Reinfelds, I., Haeusler, T., Brooks, A.J., and Williams, S. 2004. Refinement of the wetted perimeter breakpoint method for setting cease-to-pump limits or minimum environmental flows. River Res. Appl. **20**(6): 671–685. doi:10.1002/rra.784.

Rodwell, L.D., Barbier, E.B., Roberts, C.M., and McClanahan, T.R. 2003. The importance of habitat quality for marine reserve — fishery linkages. Can. J. Fish. Aquat. Sci. **60**(2): 171–181. doi:10.1139/f03-009.

Root, K.V. 1998. Evaluating the effects of habitat quality, connectivity, and catastrophes on a threatened species. Ecol. Appl. **8**(3): 854–865. doi:10.1890/1051-0761(1998)008[0854:ETEOHQ]2.0.CO;2.

Rose, K.A. 2000. Why are quantitative relationships between environmental quality and fish populations so elusive? Ecol. Appl. **10**(2): 367–385. doi:10.1890/1051-0761(2000)010[0367:WAQRBE]2.0.CO;2.

Rosenberg, A.A., Fogarty, M.J., Sissenwine, M.P., Beddington, J.R., and Shepherd, J.G. 1993. Achieving sustainable use of renewable resources. Science, **262**(5135): 828–829. doi:10.1126/science.262.5135.828. PMID:17757341.

Rosenfeld, J.S. 2003. Assessing the habitat requirements of stream

fishes: an overview and evaluation of different approaches. Trans. Am. Fish. Soc. **132**(5): 953–968. doi:10.1577/T01-126.

Rosenfeld, J.S., and Boss, S. 2001. Fitness consequences of habitat use for juvenile cutthroat trout: energetic costs and benefits in pools and riffles. Can. J. Fish. Aquat. Sci. **58**(3): 585–593. doi:10.1139/f01-019.

Rosenfeld, J.S., and Taylor, J. 2009. Prey abundance, channel structure and the allometry of growth rate potential for juvenile trout. Fish. Manag. Ecol. **16**(3): 202–218. doi:10.1111/j.1365-2400.2009.00656.x.

Roth, B.M., Kaplan, I.C., Sass, G.G., Johnson, P.T., Marburg, A.E., Yannarell, A.C., Havlicek, T.D., Willis, T.V., Turner, M.G., and Carpenter, S.R. 2007. Linking terrestrial and aquatic ecosystems: the role of woody habitat in lake food webs. Ecol. Model. **203**(3–4): 439–452. doi:10.1016/j.ecolmodel.2006.12.005.

Ryan, P.M. 1980. Fishes of the Lower Churchill River, Labrador. Fish. Mar. Serv. Tech. Rep. No. 922.

Ryder, R.A. 1982. The Morphoedaphic index — use, abuse, and fundamental concepts. Trans. Am. Fish. Soc. **111**(2): 154–164. doi:10.1577/1548-8659(1982)111<154:TMIAAF>2.0.CO;2.

Saito, L., Johnson, B.M., Bartholow, J., and Hanna, R.B. 2001. Assessing ecosystem effects of reservoir operations using food web-energy transfer and water quality models. Ecosystems (N.Y.), **4**(2): 105–125. doi:10.1007/s100210000062.

Sanger, D.M., Holland, A.F., and Gainey, C. 2004*a*. Cumulative impacts of dock shading on *Spartina alterniflora* in South Carolina estuaries. Environ. Manage. **33**(5): 741–748. doi:10.1007/s00267-004-0018-9. PMID:15503391.

Sanger, D.M., Holland, A.F., and Hernandez, D.L. 2004*b*. Evaluation of the impacts of dock structures and land use on tidal creek ecosystems in South Carolina estuarine environments. Environ. Manage. **33**(3): 385–400. doi:10.1007/s00267-003-0019-0. PMID:15031758.

Sargent, P.S., Gregory, R.S., and Schneider, D.C. 2006. Density responses of subarctic coastal marine fish and crabs to artificial reefs. Trans. Am. Fish. Soc. **135**(2): 348–360. doi:10.1577/T05-062.1.

Sayre, N.F. 2008. The genesis, history, and limits of carrying capacity. Ann. Assoc. Am. Geogr. **98**(1): 120–134. doi:10.1080/00045600701734356.

Scheuerell, M.D., Hilborn, R., Ruckelshaus, M.H., Bartz, K.K., Lagueux, K.M., Haas, A.D., and Rawson, K. 2006. The Shiraz model: a tool for incorporating anthropogenic effects and fish-habitat relationships in conservation planning. Can. J. Fish. Aquat. Sci. **63**(7): 1596–1607. doi:10.1139/f06-056.

Schindler, D.W. 2001. The cumulative effects of climate warming and other human stresses on Canadian freshwaters in the new millennium. Can. J. Fish. Aquat. Sci. **58**(1): 18–29. doi:10.1139/f00-179.

Schindler, D.E., and Scheuerell, M.D. 2002. Habitat coupling in lake ecosystems. Oikos, **98**(2): 177–189. doi:10.1034/j.1600-0706.2002.980201.x.

Schlosser, I.J. 1990. Environmental variation, life history attributes and community structure in stream fishes: implications for environmental management and assessment. Environ. Manage. **14**(5): 621–628. doi:10.1007/BF02394713.

Schlosser, I.J. 1991. Stream fish ecology: a landscape perspective. Bioscience, **41**(10): 704–712. doi:10.2307/1311765.

Sharma, R., and Hilborn, R. 2001. Empirical relationships between watershed characteristics and coho salmon (*Oncorhynchus kisutch*) smolt abundance in 14 western Washington streams. Can. J. Fish. Aquat. Sci. **58**(7): 1453–1463. doi:10.1139/f01-091.

Shvidkin, B. 1960. Ecological energy relationships at the population level. Am. Nat. **94**(876): 213–236. doi:10.1086/282124.

Smokorowski, K.E., and Derbowka, D. 2008. Methods of measuring productive capacity in Canada: summaries for review at a national workshop, October 15–16, 2007. Section 4.22, Rivers and Lakes (Hydro Québec). Can. Tech. Rep. Fish. Aquat. Sci. 2815.

Smokorowski, K.E., and Pratt, T.C. 2007. Effect of a change in physical structure and cover on fish and fish habitat in freshwater ecosystems — a review and meta-analysis. Environ. Rev. **15**: 15–41. doi:10.1139/a06-007.

Smokorowski, K.E., Withers, K.J., and Kelso, J.R.M. 1998. Does habitat creation contribute to management goals? An evaluation of literature documenting freshwater habitat rehabilitation or enhancement projects. Can. Tech. Rep. Fish. Aquat. Sci. 2249.

Smokorowski, K.E., Pratt, T.C., Cole, W.G., and Kelso, J.R.M. 2004. Experimental manipulation of habitat capacity in inland lakes. *In* Science Technology Transfer Workshop — science contributions towards improving fish habitat management. DFO Can. Sci. Advis. Sec. Proc. Ser. 2004/010. pp. 72–74.

Smokorowski, K.E., Pratt, T.C., Cole, W.G., McEachern, L.J., and Mallory, E.C. 2006. Effects on periphyton and macroinvertebrates from removal of submerged wood in three Ontario lakes. Can. J. Fish. Aquat. Sci. **63**(9): 2038–2049. doi:10.1139/f06-104.

Soulé, M.E., Estes, J.A., Miller, B., and Honnold, D.L. 2005. Strongly interacting species: conservation policy, management, and ethics. Bioscience, **55**(2): 168–176. doi:10.1641/0006-3568(2005)055[0168:SISCPM]2.0.CO;2.

Spedicato, M.T., Lembo, G., and Marmulla, G. (*Editors*). 2005. Aquatic telemetry: advances and applications. *In* Proceedings of the Fifth Conference on Fish Telemetry held in Europe, Ustica, Italy, 9–13 June 2003. FAO/COISPA, Rome, Italy.

Stalnaker, C., Lamb, B.L., Henriksen, J., Bovee, K., and Bartholow, J. 1995. The instream flow incremental methodology: a primer for IFIM. U.S. Department of the Interior, National Biological Service, Washington, D.C., Biological Report 29.

Sutherland, W.J. 1996. From individual behaviour to population ecology. Oxford University Press, Oxford, UK.

Tennant, D.L. 1976. Instream flow regimes for fish, wildlife, recreation, and related environmental resources. Fisheries (Bethesda), **1**(4): 6–10. doi:10.1577/1548-8446(1976)001<0006:IFRFFW>2.0.CO;2.

Tharme, R.E. 2003. A global perspective on environmental flow assessment: emerging trends in the development and application of environmental flow methodologies for rivers. River Res. Appl. **19**(5–6): 397–441. doi:10.1002/rra.736.

Tyler, J.A., and Rose, K.A. 1994. Individual variability and spatial heterogeneity in fish population models. Rev. Fish Biol. Fish. **4**(1): 91–123. doi:10.1007/BF00043262.

U.S. Fish and Wildlife Service. 1980. Habitat evaluation procedure (HEP). U.S. Fish and Wildlife Service, Division of Ecological Services, Washington, D.C., USFWS ESM 102.

U.S. Fish and Wildlife Service. 1981. Standards for the development of habitat suitability index models. U.S. Fish and Wildlife Service, Division of Ecological Services, Washington, D.C., USFWS ESM 103.

Van Horne, B. 1983. Density as a misleading indicator of habitat quality. J. Wildl. Manage. **47**(4): 893–901. doi:10.2307/3808148.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., and Cushing, C.E. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. **37**(1): 130–137. doi:10.1139/f80-017.

Vélez-Espino, L.A., and Koops, M.A. 2009*a*. Recovery potential assessment for lake sturgeon in Canadian designatable units. N. Am. J. Fish. Manage. **29**: 1065–1090. doi:10.1577/M08-034.1.

Vélez-Espino, L.A., and Koops, M.A. 2009*b*. Quantifying allowable harm in species at risk: application to the Laurentian black

redhorse (*Moxostoma duquesnei*). Aquat. Conserv. **19**: 676–688. doi:10.1002/aqc.1023.

Vilizzi, L., Copp, G.H., and Roussel, J.M. 2004. Assessing variation in suitability curves and electivity profiles in temporal studies of fish habitat use. River Res. Appl. **20**(5): 605–618. doi:10.1002/rra.767.

Walters, C., Pauly, D., and Christensen, V. 1999. Ecospace: prediction of mesoscale spatial patterns in trophic relationships of exploited ecosystems, with emphasis on the impacts of marine protected areas. Ecosystems (N.Y.), **2**(6): 539–554. doi:10.1007/s100219900101.

Wang, L., Lyons, J., Kanehl, P., and Gatti, R. 1997. Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. Fisheries (Bethesda), **22**: 7–12.

Williams, J.G. 1999. Stock dynamics and adaptive management of habitat: an evaluation based on simulations. N. Am. J. Fish. Manage. **19**(2): 329–341. doi:10.1577/1548-8675(1999)019<0329:SDAAMO>2.0.CO;2.

Yee, D.A., Yee, S.H., Kneitel, J.M., and Juliano, S.A. 2007. Richness–productivity relationships between trophic levels in a detritus-based system: significance of abundance and trophic linkage. Oecologia (Berl.), **154**(2): 377–385. doi:10.1007/s00442-007-0837-5. PMID:17713787.

CORRIGENDUM / RECTIFICATIF

Corrigendum: Data weighting in statistical fisheries stock assessment models

R.I.C. Chris Francis

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In Appendix A of the published article, there were some errors associated with the equation, and associated derivation, of method TA1.10. The equation for this method (last line of Table A1) should be

$$\mathrm{Var}_y\left[(\overline{O}_{fy} - \overline{E}_{fy})/(S_2 + \overline{E}_{fy}^2 S_3 - 2\overline{E}_{fy} S_4)^{0.5}\right] = 1$$

and the associated footnote should be "[6]See text for definitions of S_2, S_3, and S_4."

Also, the third sentence of the last paragraph of the section headed **Methods allowing for correlations** should be as follows: "Thus we treat $\overline{O}_{fy}$ as being equal to $\sum_b x_b O_{fby} / \sum_b O_{fby}$ and calculate its variance using the standard approximation for the variance of a ratio of two random variables (eq. 10.17 of Stuart and Ord 1987), which produces $\mathrm{Var}(\overline{O}_{fy}) = S_2 + \overline{E}_{fy}^2 S_3 - 2\overline{E}_{fy} S_4$, where $S_2 = \sum_b (x_b c_{fby} E_{fby})^2$, $S_3 = \sum_b (c_{fby} E_{fby})^2$, and $S_4 = \sum_b x_b (c_{fby} E_{fby})^2$."

Reference

Stuart, A., and Ord, J.K. 1987. Kendall's advanced theory of statistics. Vol. 1. Distribution theory. Charles Griffin and Company Limited, London, UK.

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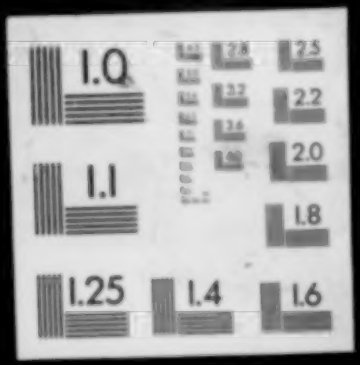
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